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(54) Title: GENERATION OF COMBINATORIAL LIBRARIES OF COMPOUNDS CORRESPONDING TO VIRTUAL LIBRARIES OF COMPOUNDS

(57) Abstract

The present invention provides methods for the generation of virtual libraries of compounds and using these libraries to build combinatorial libraries. The virtual compounds are generated *in silico*. The present invention encompasses methods for tracking the addition of fragments, use of reagents, and transformations performed. Further, methods for interfacing the information necessary to generate libraries of compounds with instrumentation that conducts the actual synthesis of the compounds are provided.

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GENERATION OF COMBINATORIAL LIBRARIES OF COMPOUNDS CORRESPONDING TO VIRTUAL LIBRARIES OF COMPOUNDS

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of U.S. Serial No. 09/076,214 filed May 12, 1998, which claims priority to provisional U.S. Serial No. 60/085,092 filed May 12, 1998, each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention is directed to methods for the generation of virtual combinatorial libraries of small molecules and other ligands. The members or molecules of the combinatorial libraries are generated *in silico*, and are designed to bind to identified target molecules *in silico*. The present invention also includes methods for docking the library members to desired target molecules whereby the library members are bound to such targets *in silico*. The present invention also encompasses methods for interfacing the synthetic information generated *in silico* with instrumentation such as a parallel array synthesizer which conducts the actual synthesis of desired members of the combinatorial libraries.

BACKGROUND OF THE INVENTION

Combinatorial chemistry is a recent addition to the toolbox of chemists and represents a field of chemistry dealing with the synthesis of a large number of chemical entities. This is generally achieved by condensing a small number of reagents together in all combinations defined by a given reaction sequence. Advances in this area of chemistry include the use of chemical software tools and advanced computer hardware which has made it possible to

consider possibilities for synthesis in orders of magnitude greater than the actual synthesis of the library compounds. The concept of "virtual library" is used to indicate a collection of candidate structures that would theoretically result from a combinatorial synthesis involving reactions of interest and reagents to effect those reactions. It is from this virtual library that compounds are selected to be actually synthesized.

Project Library (MDL Information Systems, Inc., San Leandro, CA) is said to be a desktop software system which supports combinatorial research efforts. (*Practical Guide to Combinatorial Chemistry*, A. W. Czarnik and S. H. DeWitt, eds., 1997, ACS, Washington, D.C.) The software is said to include an information-management module for the representation and search of building blocks, individual molecules, complete combinatorial libraries, and mixtures of molecules, and other modules for computational support for tracking mixture and discrete-compound libraries.

Molecular Diversity Manager (Tripos, Inc., St. Louis, MO) is said to be a suite of software modules for the creation, selection, and management of compound libraries. (*Practical Guide to Combinatorial Chemistry*, A. W. Czarnik and S. H. DeWitt, eds., 1997, ACS, Washington, D.C.) The LEGION and SELECTOR modules are said to be useful in creating libraries and characterizing molecules in terms of both 2-dimensional and 3-dimensional structural fingerprints, substituent parameters, topological indices, and physicochemical parameters.

Afferent Systems (San Francisco, CA) is said to offer combinatorial library software that creates virtual molecules for a database. It is said to do this by virtually reacting precursor molecules and selecting those that could be actually synthesized (Wilson, *C&EN*, April 27, 1998, p.32).

While only Project Library and Molecular Diversity Manager are available commercially, these products do not provide facilities to efficiently track the reagents employed for the introduction of fragments into the desired compounds being generated. Further, these products are unable to track mixtures of compounds that are generated by the introduction of multiple fragments by the use of multiple reagents. Therefore, it is desirable to have available methods for handling mixtures of compounds, as well as methods for the tracking of chemical reactions or transformations utilized in the synthesis of individual compounds and mixtures thereof.

SUMMARY OF THE INVENTION

In accordance with the present invention, there are provided methods for the generation of virtual combinatorial libraries of small molecules. These library molecules or members are generated *in silico*. Library members of larger molecular weight, such as those that are polymeric in nature, may also be generated using the methods of the present invention.

The present invention further provides methods for tracking and maintaining in databases, the fragments, reagents and unique combinations of these used for the *in silico* generation of the library members. Methods for interfacing the information necessary for the generation of libraries *in silico,* as instructions designed to direct the actual synthesis of the library members on an instrument such as a parallel array synthesizer, are also provided in the present invention.

While there are a number of ways to identify molecular interaction sites, identify compounds likely to interact with molecular interaction sites of RNA and other biological molecules, synthesize such compounds and analyze their binding, preferred methodologies are described in U.S. Serial Numbers 09/076,440, 09/076,405, 09/076,447, 09/076,206, 09/076,214, and 09/076,404, each of which was filed on May 12, 1998 and each assigned to the assignee of this invention. All of the foregoing applications are incorporated by reference herein in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a compound, compound CI, dissected into its constituent fragments;

Figure 2 shows the various identifying characteristics of the fragments comprising compound CI;

Figure 3 shows the various identifying characteristics of the reagents used to introduce the corresponding fragments comprising compound CI;

Figure 4 is a list of transformations that link the fragments and reagents associated with the generation of compound CI;

Figure 5 is a schematic for the introduction of a common fragment using two different reagents;

Figure 6A is a schematic for the use of a single reagent for the introduction of two

different fragments into a compound;

Figure 6B is a schematic showing the use of a common reagent for the introduction of a common fragment into the compound which can further be converted into two different fragments within the compound generated;

Figure 7 shows the symbolic addition of fragments yielding a symbolic compound, compound CI';

Figure 8 is a symbolic reagent table;

Figure 9 is a symbolic fragment table;

Figure 10 is a symbolic transformation table;

Figure 11 shows the generation of individual compounds, compounds C1 and C4, and a mixture, mixture M1;

Figure 12 shows the generation of further mixture, mixture M2;

Figure 13 shows the generation of an additional mixture, mixture M3;

Figures 14A and 14B show the generation of an additional mixture, mixture M4;

Figure 15 shows tables for tracking compound C1 by the fragments added and or transformations performed;

Figure 16 shows tables for tracking mixture M1 by the transformations performed;

Figure 17 shows tables for tracking mixture M2 by the transformations performed;

Figure 18 shows tables for tracking mixture M3 by the transformations performed;

Figure 19 is a pictorial elevation view of an apparatus used to robotically synthesize compound;

Figure 20 is a pictorial plan view of an apparatus used to robotically synthesize compounds;

Figure 21 is a first synthetic reaction scheme for preparing a library of compounds; and

Figure 22 is a second synthetic reaction scheme for preparing the library of compounds of Figure 21.

The present invention is directed to computational methods employed for the *in silico* design and synthesis of combinatorial libraries of small molecules. The library members are generated *in silico*. The present invention also encompasses methods for tracking and storing the information generated during the *in silico* creation of library members into relational databases for later access and use of this information to synthesize chemical compounds

corresponding to those generated *in silico*. For the purposes of this specification, *in silico* refers to the creation in a computer memory, i.e., on a silicon or other like chip. Stated otherwise *in silico* means "virtual."

According to the methods of the present invention, each compound or library member is dissected into its component or constituent parts referred to as fragments. Thus each compound that is generated is considered to be comprised of constituent fragments such that the sum of the molecular formulas of each of the fragments when added together totals the molecular formula of the compound generated. This dissection can be done in a variety of ways using chemical intuition. Thus a variety of components of fragments may be identified, each of which lend themselves to readily available reagents or reactions to generate diverse compounds. Further, each fragment is associated with at least one reagent, which represents the necessary chemical to be used to introduce that desired fragment into the compound being generated *in silico*. Dissection of compounds is based on the ease of synthesis of the reagents, commercial availability of the reagents, or a combination of both. Each of the fragments and reagents are stored in a relational database and are described in terms of identifying characteristics in the database. A fragment may be available from a variety of starting materials or reaction schemes. So when a library is being generated, which entails building a database, the fragments used in building that library can be stored in the database using the corresponding set of reagents and reaction conditions. When another library is to be generated, the fragment information stored in the database is now available for use in the generation of the new library of compounds. Similarly, when a third library is being generated, an even greater quantity of fragment, reagent, and reaction information is available in the database. Thus the methods of the present invention represent a dynamic method of building a database associated with building libraries of compounds. Initial library generation requires database input for fragments, reagents and transformations necessary for desired library. As the database grows, however, an increasing number of fragments and reagents are available in the database, which simplifies the generation of subsequent libraries of compounds and makes for more routine combinatorial synthetic efforts which can be accomplished with increasing ease and efficacy.

Fragments that are recorded in the database may be defined using identifying characteristics. Identifying characteristics defining fragments include a structural

representation (as a 2-dimensional or 3-dimensional file), name, molecular weight, molecular formula, and attachment points or nodes (which denote sites of attachment or linkage of the fragment to other fragments of the compound being generated *in silico*). For the purpose of describing this invention, 2-dimensional representations are used, which are further simplified by the use of symbolic representations without reference to any particular chemical entities. The symbolic representations as used herein merely shows how fragments can be tracked to further the methods of the present invention. Other identifying characteristics may also be added to the database. Any characteristic that is desired to be tracked may be included in the database, including biological data, chemical reactivity rates, or other physical or chemical properties. Further, a fragment may also be created by modifying a reagent, and such modifications can be added to the database in terms of changes made to the reagent structure. Some of the identifying characteristics associated with any fragment may be common to those of the corresponding reagent. The related fragment thus created can then be stored in the relational database.

Identifying characteristics defining reagents include a structural representation, name, molecular weight, molecular formula, and source, such as a commercial source or a unique compound defined by the user. In case of a commercial source for the reagent, a catalog number or a link to a web page can be provided. Some commonalities may exist between the identifying characteristics associated with a reagent and those associated with the related fragment.

Further, in accordance with the present invention, a compound is the sum of various transformations. Transformation is the nomenclature attributed according to the present invention to a chemical synthesis. A transformation is a 1:1 link between a fragment and a reagent. Thus each transformation describes a unique conversion of a reagent into the corresponding fragment as introduced into a compound. When the compound being generated *in silico* is broken down into its component fragments, and the corresponding reagents have been identified, each fragment is linked to the corresponding reagent in a 1:1 relationship in order to describe a transformation. Thus, according to the present invention, a transformation may be viewed as the source of a fragment, thereby linking that fragment to a particular synthetic method or reaction. This description of a transformation according to the methods of the present invention also includes any auxiliary reagents or conditions used to effect the

reaction denoted by the transformation, such as temperature and pressure requirements, catalysts, activators, solvents, or other additives.

Each combination of a fragment and reagent in a 1:1 link comprises a different transformation. Therefore, each transformation is unique. The present invention allows the tracking of fragments in terms of the reaction or transformation in which those fragments are introduced into the compounds of the library. Thus the database describes not only the compounds generated in terms of their constituent fragments, but also in terms of the synthetic pathways to produce those compounds, *i.e.* the related transformations to generate the library compounds. In this manner, a user of the present invention can generate a virtual library of compounds by simply selecting the fragments desired. Alternately, a user can also generate the compounds by selecting the chemical pathways required for actual synthesis of the compounds. This is accomplished by selecting the appropriate transformation associated with the generation of the desired compounds. Here, the user uses intuition or an in silico expert system to assist in selecting those transformations that are expected to allow generation or synthesis of the desired compounds. Each of the transformations created *in silico* is stored in the relational database and described in terms of identifying characteristics. Identifying characteristics defining transformations include the fragment, the reagent, and any auxiliary reagent or conditions necessary to effect the conversion of the reagent into the fragment as incorporated into the compound.

For example, consider in Figure 1 the *in silico* generation of compound CI according to the methods of the present invention. As shown in Figure 1, upon dissection of CI (molecular formula of $C_{12}H_{18}N_2O_5S_1$), its constituent fragments can be denoted as F_i (molecular formula of H_2NO), F_{ii} (molecular formula of C_5H_9NO), and F_{iii} (molecular formula of $C_7H_7O_3S$). F_i can also be a hydroxyl amine moiety linked to a solid support, *i.e.* P-O-NH, wherein P is a solid support. The sum of the molecular formulas of each of the fragments totals the molecular formula of compound CI.

As shown in Figure 2, each of the fragments, F_i, F_{ii}, and F_{iii}, are stored in a relational database, and are described in terms of identifying characteristics including a structural representation (which may be 2-dimensional or 3-dimensional), an identifier or name, molecular formula and attachment points or nodes which signify sites on the fragment which are linked to other fragments in compound CI. Other information such as molecular weight

can also be associated with the fragment in the database.

As shown in Figure 3, each of the corresponding reagents (R_i, R_{ii}, and R_{iii}) are also stored in the relational database, and described in terms of identifying characteristics. Identifying characteristics used to define the reagents include a structural representation, and identifier or name and molecular formula. As with the fragment, other associated information such as molecular weight and source (such as a commercial source verses user-supplied, amount on hand, special handling, etc.) can also be stored in database in association with the individual reagents.

Next, each of the transformations associated with the *in silico* generation of compound CI are also stored in the relational database. As shown in Figure 4, transformation T_i links reagent R_i with fragment F_i, T_{ii} links R_{ii} with F_{ii}, and T_{iii} links R_{iii} with F_{iii} in a 1:1 relationship. Also, associated with each transformation is the necessary reaction condition, so that transformation T_i is associated with reaction condition alpha, T_{ii} with reaction condition beta, and T_{iii} with reaction condition gamma. In the case of transformation T_{iii}, reagent R_{iii} may be a hydroxyl amine attached to a solid support so that fragment F_{iii} can be represented as a hydroxyl amine moiety attached to a solid support.

While each fragment may be arrived at or generated by a unique corresponding reagent, the present invention also encompasses common fragments that may be generated via two or more reagents, so that two or more transformations can lead to the same fragment. As shown in Figure 5, the common fragment CH_3-CH_2-C(=O)- may be arrived at via transformation A, which employs reagent X (an acid chloride), CH_3-CH_2-C(=O)Cl. The common fragment can also be introduced into a compound being generated *in silico* via transformation B, which employs reagent Y (an acid anhydride), CH_3-CH_2-C(=O)-O-C(=O)-CH_2-CH_3. Therefore, in accordance with the methods of the present invention, a common fragment can be introduced into the compound via two or more different reagents, and thus via two or more distinct transformations.

Alternately, a common reagent may be employed to effect two or more conversions forming two or more different fragments. This then represents two or more different transformations associated with different conditions. For example, as shown in Figure 6a, common reagent Z, CH_3-CH_2-NH_2, can be employed to introduce an alkene fragment into the compound under conditions favoring Schiff's base formation. This represents transformation

X. The same common reagent Z, however, can also be employed to introduce an amide fragment into the compound by using a different set of conditions, constituting transformation Y. Thus, a common reagent can introduce two or more different fragments into final compounds being generated *in silico*, and can be associated with two or more transformations depending upon the conditions associated with each of those transformations.

Additionally, once a fragment has been introduced into a compound, it can be further modified and converted into yet another fragment without effecting any other chemical changes within the compound formed. As an example, shown in Figure 6b, consider common reagent Z', CH_3-CH_2-C(=O)CH_2-Cl. Common reagent Z' corresponds to a fragment having the structure CH_3-CH_2-C(=O)CH_2-. Common reagent Z' may be used to introduce an alkene fragment into the final compound, representing transformation X', under conditions favoring reduction and dehydration. Common reagent Z', however, can also be used to introduce a hydroxyalkyl fragment into the final compound under conditions favoring reduction. This represents transformation Y'.

The present invention may be described more generally, in terms of symbolic representations. Symbolic representations are used to describe the methods of the present invention because such representations are not limited to any particular chemistry. Symbolic representations merely denote the manner of using the present invention with multiple chemical entities. Each symbol used in the representations describing the present invention may represent one compound or multiple compounds because the present invention is not limited to tracking a single compound, but may be used to track a vast variety of compounds that can be generated.

Figure 7 shows the symbolic addition of fragments which yields compound CI'. The fragments have structures $F_{i'}$, $F_{ii'}$, and F_{iii} that are added sequentially to yield compound CI'. Structures $F_{i'}$, $F_{ii'}$, and $F_{iii'}$ are symbolic representations of the fragments that constitute compound CI'. These fragments can be stored in the relational database with the corresponding identifying characteristics for each of them, including the structural representation, name, molecular formula, and attachment sites or nodes. A visual inspection of compounds C1 and C1' revels the commonality between the chemical compound C1 and the symbolic representation of a compound C1' as well as the chemical structure of the fragments and the symbolic structure of the fragments.

A symbolic reagent table is shown in Figure 8. Reagents R1 to R10 can be described in terms of their structure, name, molecular formula, molecular weight, and source as well as other information that might be desired to be associated with the reagents.. R3 and R4 are two different reagents, but may be used to introduce the same fragment into a compound. This depends upon the reaction conditions used as reagent R3 is used in a transformation associated with one set of conditions, while reagent R4 is used in another transformation associated with a different set of conditions. Also, reagent R5 is comprised of a mixture of two reagents or components. These may be (R)- and (S)-stereoisomers, D- and L-isomers, or may be two completely different reagents. While R5 here is represented as a mixture of only two reagents or components, it will be recognized by the art-skilled that the methods of the present invention may be practiced using a mixture of two or more reagents. Typical reagent mixtures used in constructing libraries might have four, five or more individual reagent constituting the mixture.

Figure 9 shows a symbolic fragment table. Fragments F1 to F8 are stored in the relational database with identifying characteristics that include a structural representation, name, molecular weight, molecular formula, and attachment sites or nodes. This table depicts symbolic representations of the various fragments that are introduced into the compounds of the library by the use of reagents symbolized in Figure 8. Thus it can be seen that fragment F1 can be introduced into the compound by employing reagent R1. In fragment F1, X is an identifier for an attachment site. This indicates that X is the site at which F1 attaches to another fragment in a compound. Similarly, fragment F2 may be introduced into a compound (attaching at its X site) by employing reagent R2.

Fragment F3, however, can be introduced into the compound by the use of either reagent R3 or R4. This allows for selection in the choice of the reagent used, and also allows for the consideration of the compatibility of the chemistries involved in the introduction of other fragments into the compound. Next, fragment F4 (which is a mixture of fragments) can be introduced via the use of reagent R5, which is a mixture of reagents, as shown in Figure 8.

Fragment F5 has two attachment sites, indicating that other fragments can attach at sites X and Y when F5 has been incorporated into a compound. The presence of two attachment sites indicates that two attachments may be undertaken to build a compound when

dealing with F5. Here again, as before, F5 can be introduced into the compound using either of reagents R6 or R7, depending upon the reaction conditions used and the chemistries involved when introducing other fragments to build the compound.

Fragments F7 and F8 can be introduced into a compound being created *in silico* by employing reagents R9 and R10, respectively. Both these fragments have three attachment sites, indicating that three attachments to other fragments can occur when using these fragments to build a compound *in silico*. While fragments F7 and F8 have three attachment sites, it is recognized by the art-skilled that more than three attachment sites may be present in a fragment, allowing for more attachments to the fragment upon introduction into a compound (with the use of an appropriate reagent).

With the fragment and reagent tables in place in the relational database, a transformation table is created in accordance with the methods of the present invention, by linking a fragment with a reagent to form a unique transformation. Figure 10 shows a symbolic transformation table where a fragment is linked to a reagent in a 1:1 relationship. The identifying characteristics describing each transformation include a 1:1 link (a one to one link) between a fragment and a reagent, and the reaction conditions which include, solvent, concentration, temperature and pressure requirements, or auxiliary reagents necessary to effect the introduction of the fragment into the compound by using an appropriate reagent. Auxiliary reagents include catalysts, activators, acids, bases or other chemicals or additives necessary to effect the fragment introduction described. For example a base can always be added with an alkyl halide to scavenge the acid generated with use of the alkyl halide.

As seen in Figure 10, transformation T1 links fragment F1 with reagent R1. T1 also specifies the reaction conditions (α) associated with this 1:1 link. Similarly, T2 links F2 with R2 under conditions β. Transformations T3 and T4 are each unique transformations despite being associated with a common fragment, F3. Transformation T3 links common fragment F3 with reagent R3 under conditions α, while transformation T4 links the common fragment F3 with another reagent, R4, under the different conditions, conditions δ. For example reagent R3 might be an alkyl chloride while R4 might be an alkyl iodide. While these reagents are similar (they are both alkyl halides), they might be used under different reaction conditions. Use of different reagents to effect the introduction of the same fragment into the compound being generated *in silico* represents two unique transformations. This indicates two distinct

or unique synthetic ways of introducing the same fragment into the compound. Depending upon the totality of the chemical steps involved in synthesizing the compound, one transformation may be preferred over other transformations that introduce the same fragment into the compound.

Transformation T5 links fragment F4 with reagent R5. R5 is a mixture of reagents, such as (R)- and (S)-stereoisomers, D- and L-isomers, or two or more different reagents. As a result, use of R5 leads to the introduction of a mixture of fragments F4 into the compound. The art-skilled will recognize that the multiple reagents in R5 are selected such that they are capable of being mixed together, do not react with each other, and react under similar reaction conditions. For example, R5 may be comprised of a mixture of acid halides. These do not react with each other, but do react similarly with a nucleophile under similar conditions. It is also recognized by the art-skilled that a reagent is not limited to only one or two components or constituent reagents, but in fact may comprise of two, three, four, five or more reagents or components.

When using a mixture of reagents, each of the individual component reagents may have different chemical reactivity rates. If a correction is not made for this, this could result in their products being unequally represented in the product compounds. This is solved by adjusting the concentration of each reagent in the reaction mixture relative to the other reagents in the mixture such that the relative rates are the same. This is effected by comparing to the reactivity of each of the reagents to a chosen standard reagent. The standardized reactivity rates can then be used to adjust the concentration of each constituent reagent in the reagent mixture to compensate for the varied reaction rates. Thus a mixture of reagents with different reaction rates may be used in one reagent mixture to still generate equivalent quantities of the desired compounds in the library.

Transformations T6 and T7 are similar to transformations T3 and T4 except that conditions identifying each of these transformations are different. Transformation T6 links fragment F5 with reagent R6 under conditions ε, while transformation T7 links the same fragment F5 with a different reagent R7 under different conditions (condition α). As the conditions associated with transformations T6 and T7 are different, this allows selection of compatible chemistries with other fragments during any particular synthesis being used. This is a very useful and very important consideration in actually synthesizing real libraries. When

it is desired to introduce fragment F5 into the compound, the actual chemistries used to build the compound can be initially be considered in selecting transformation T6 or T7, and thus reagents R6 or R7. This is in direct opposition to any chemical database generator that only considers the compound structure not the actual chemistries necessary to build a compound.

Transformations T9 and T10 link fragment F7 with reagent R9 and fragment F8 with reagent R10, respectively. Both transformations are identified to be associated with reaction conditions γ. Fragments F7 and F8 have three attachment sites, but it is recognized that these fragments may have more than three attachment sites, thereby increasing the complexity of the compounds generated, and increasing the number of rounds that may be employed to attach other fragments. For the three sites illustrated, if three sets of different reagent mixtures each have five reagents in the set are used, then 125 compounds will be generated for fragment F7 and a further 125 compounds will be generated for fragment F8.

The methods of the present invention may be used to generate single compounds or mixtures of compounds. A mixture comprises two or more compounds and may involve the use of two or more reagents (thus introduction of two or more fragments) at the outset of library generation, introduction of a mixture of reagents (thus a mixture of fragments) at a subsequent stage of library generation, or a combination of both such techniques. Figures 11 and 12 illustrate this aspect of the present invention.

As shown in Figure 11, the methods of the present invention may be used to generate single compounds such as C1 and C4, or may also be used to generate a mixture of compounds, M1, comprising compounds C2 and C3. Library generation commences with selecting fragment F7 (with three attachment sites), in the first round (*i.e.* round n). In the second synthesis round (*i.e.* round n+1), F7 is combined with fragment F2, constituting synthetic pathway P1a, and resulting in the formation of complex fragment CF1. F7 possesses three attachment sites (*i.e.* X, Y and Z). Thus round n+1 will not be complete until each of X, Y and Z have been used, if desired, to attach other fragments to. Stepping around each of X, Y and Z, and attaching fragments to these sites, occurs in that sequential order. Once sites X, Y and Z of the fragment selected in the first synthesis round (*i.e.* round n) have been exhausted, stepping around the attachment sites present in the next added fragment constitutes the next synthesis round (*i.e.* the third synthesis round, or round n+2). Here again, when all desired attachment sites on this fragment have been used, that particular synthesis round is

complete. This attachment iteration around the desired and available attachment sites of the fragments added continues until the desired compounds have been generated.

As shown in Figure 11, CF1 is next subjected to synthetic pathway P1b wherein fragment F1 is introduced into CF1, thereby forming complex fragment CF2. CF2 is then subjected to synthetic pathway P1c wherein fragment F5 is added to CF2, leading to the formation of complex fragment CF3. This completes synthesis round n+1 (*i.e.* the second round of fragment introduction, or synthesis, to build the compound). As fragment F5 has two attachment sites, CF3 has an available attachment site (*i.e.* site Y). Introduction of fragments to this site (Y site) constitutes synthesis round n+2 (*i.e.* the third round) because all the desired attachment sites on the previously added fragment have been exhausted. Next, CF3 is subjected to synthetic pathway P2 wherein fragment F4 is introduced into CF3 at attachment site Y. As F4 is a mixture of two components, a mixture (M1) of two compounds, C2 and C3, is generated.

A single compound, however, may also be generated using the present scheme of fragment introduction. Thus, compound C1 can be generated by subjecting CF3 to synthetic pathway P1d wherein CF3 is combined with fragment F3, which attaches to site Y in CF3. The introduction of fragment F3 into CF3 constitutes the third synthesis round (*i.e.* round n+2), leading to the generation of C1.

Alternately, CF3 can be subjected to synthetic pathway P3a wherein fragment F6 is introduced into CF3 to form CF4. This represents the third synthesis round (*i.e.* round n+2). CF4 has one more available attachment site (*i.e.* site Y) to which fragment F2 may be attached via synthetic pathway P3b. This leads to the generation of compound C4 which is a compound of increased complexity because of the number of attachment sites on the chosen fragments and synthetic pathways employed. The addition of fragment F6 to CF4 constitutes the third synthesis round (*i.e.* round n+2). Addition of fragment F2 to CF4 represents the fourth synthesis round, or round n+3, because P3b involves addition of a fragment (fragment F2) onto a site (*i.e.* site Y in CF4) which has been generated by adding fragment F6 to CF3, thus exhausting the available attachment sites on the previously added fragment in CF4 (*i.e.* fragment F5). That is, the addition of fragment F6 completed round n+2 (or the third synthesis round) because F6 attached to the last available attachment site on CF3 (*i.e.* site Y in CF3).

For the reactions effected at path P1c in Figure 11, a single fragment (F5) can be added to CF2 via use of either reagents R6 or R7 (as thus via the transformations associated with R6 and R7). While these additions are represented as two unique transformations for the purpose of tracking in the database on the invention, these additions in effect perform the same chemical conversion. Thus, the simultaneous tracking of compounds generated according to the methods of the invention is useful not only in working with virtual libraries of compounds, but also provide the user with a choice of synthetic pathways along which the compounds can be actually synthesized. This tracking aspect of the present invention is, therefore, a novel and unique way to account for the fragments being introduced, the related transformations (or reactions) associated with the fragments, and the alternate transformations that lead to the introduction of a common fragment into the desired compounds. The present invention allows not only the tracking of individual compounds that are generated by the use of multiple reagents, but also allows for the simultaneous tracking of multiple compounds that are generated via multiple transformations. While the methods described herein represent the tracking aspects of the invention in terms of symbolic representations or tables, it is recognized by the art-skilled that a variety of computer algorithmic codes and techniques may be employed for the individual or simultaneous tracking aspects described above.

The present invention further provides methods for the one-pot generation of mixtures of compounds by commencing the library generation using different starting fragments in a one-pot fashion. One-pot generation or synthesis of compounds refers to the formation of multiple compounds in a single reaction vessel (*i.e.* one pot). This is possible if compatible chemistries are selected. Examples of such single vessels include but are not limited to multiple well plates, e.g. a 96-well plate, reactions flasks, e.g. a 25 mL flask, or even an industrial reactor. The reactions, or transformations, are performed in one vessel regardless of the size of the reaction vessel. The concept of one-pot synthesis is irrelevant to the generation of virtual libraries of compounds as these virtual libraries are merely generated *in silico*. The concept of one-pot synthesis becomes relevant, however, when the actual synthesis of libraries of compounds is to be undertaken. Thus the compounds can be tracked separately for compound building in order to generate distinct chemical structures, however, they can be group together for synthesis allowing them to be made in the same "pot."

An example of a one-pot synthesis was shown in Figure 11 with the addition of the

complex reagent R5 to form mixture M1. A further one-pot synthesis is shown in Figure 12, where a further mixture of compounds is generated. Mixture M2 comprising compounds C1 and C5 can be generated by starting with fragments F7 and F8 in the first synthesis round (*i.e.* round n). Each of these fragments have three attachment sites onto which other fragments can be introduced. As a result, subjecting the two fragments to synthetic pathway P1a wherein F7 and F8 are combined with fragment F5 at site X, results in the one-pot formation of complex fragments CF1 and CF5. CF1 and CF5 are next subjected to synthetic pathway P1b wherein fragment F1 is introduced into CF1 and CF5 at site Y, thereby forming complex fragments CF2 and CF6. CF2 and CF6 are next subjected to synthetic pathway P1c wherein fragment F5 is introduced into these complex fragments at site Z, forming CF3 and CF7. This completes the second synthetic round (*i.e.* round n+1). As fragment F5 contains two attachment sites, after introduction into CF3 and CF7, there is still available an attachment site (*i.e.* site Y) for further introduction of another fragment. Thus CF3 and CF7 are converted to a mixture (M2) of compounds C1 and C5 via synthetic pathway P1d wherein CF3 and CF7 are combined with fragment F3 which attaches to the Y site on fragment F5 in CF3 and CF7. The introduction of fragment F3 at site Y in CF3 and CF7 represents the third synthetic round (*i.e.* round n+2).

Yet another symbolic example of the one-pot generation of mixtures of compounds, in accordance with the present invention, is shown in Figure 13. *In silico* generation of compounds commences with the selection of fragment F7, which has three sites of attachment (X, Y, and Z). This represents the first synthesis round (*i.e.* round n). Next, F7 is subjected to synthetic pathway P1a wherein F7 is combined with fragment F2. F2 attaches to site X on fragment F7, forming complex fragment CF1. At this stage, CF1 is subjected to two synthetic pathways, P1b and P1b'. P1b employs fragment F1 which is introduced onto site Y on CF1, thereby forming complex fragment CF2, while P1b' employs fragment F3 which is introduced onto site Y on CF1, thereby forming complex fragment CF8. Thus a mixture of complex fragments (CF2 and CF8) are formed. Both fragments, F1 and F3 can be introduced together (such as from a single reagent bottle when actual synthesis is being undertaken) for the one-pot generation of compounds if the chemistries associated with introduction of these fragments into the compounds are compatible. If not, these fragments can be introduced separately. Next, CF2 and CF8 are subjected to synthetic pathway P1c wherein both complex

fragments are combined with fragment F5 which attaches to site Z on CF2 and CF8, thereby forming complex fragments CF3 and CF9. The formation of CF3 and CF9 completes the second synthesis round (*i.e.* round n+1). As fragment F5 has two sites of attachment, site Y is still available for attachment to another fragment. Therefore, CF3 is subjected to synthetic pathway P3 wherein CF3 is combined with fragment F4. Introduction of F4 represents the third synthesis round (*i.e.* round n+2). F4 is a mixture of fragments (and introduced by adding a mixture of reagents), as shown in Figure 9. As a result, synthetic pathway P2 leads to the generation of compounds C2 and C3. Simultaneously, CF9 combines with fragment F4, via synthetic pathway P2', leading to the generation of compounds C7 and C8. Thus mixture M3 is formed comprising compounds C2, C3, C7 and C8.

The present invention also provides methods for the generation of increasingly complex mixtures of compounds. An example is shown in Figures 14a and 14b where mixture M4 is generated and comprises sixteen compounds. The compounds in mixture M4 can be generated by starting with fragments F7 and F8 in the first synthesis round (*i.e.* round n). These fragments can then be combined with fragment F2, which is introduced at site X in each of F7 and F8, forming complex fragment CF1 and CF5. Following this, a mixture of fragments F1 and F3 are introduced into CF1 and CF5 at site Y of these complex fragments, leading to the formation of four complex fragments, CF2, CF6, CF8 and CF11. These complex fragments are next combined with a mixture of fragments F5 and F6. Both F5 and F6 have two attachment sites such that site X on F5 and F6 attaches to site Z on CF2, CF6, CF8 and CF11 forming a mixture of eight complex fragments, CF3, CF7, CF9, CF12, CF13, CF14, CF15 and CF16. This completes the second synthesis round (*i.e.* round n+1). As fragments F5 and F6 have two attachment sites, X and Y, the abovementioned eight complex fragments have one more available attachment site (*i.e.* site Y) onto which another fragment may be introduced. Attachment of a fragment to site Y on these eight complex fragments represents the third synthesis round (*i.e.* round n+2). Next, fragment F4 is introduced into CF3, CF7, CF9, CF12, CF13, CF14, CF15 and CF16. As fragment F4 is a mixture of two constituent fragments, sixteen compounds are generated: C2, C3, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19 and C20. Thus it can be seen that by using multiple fragments in a one-pot fashion and combining with mixtures of fragments, mixtures of compounds of increasing complexity can be generated. The example in Figures 14a and 14b

shows sixteen unique compounds being generated as mixture M4 when the library is generated by starting with two fragments. It is recognized by the art-skilled that if the library generation is commenced with more than two fragments or multiple fragments are added to the same precursor fragment, even more complex mixtures of compounds can be generated.

The present invention also provides methods for keeping track of fragment addition in the various synthesis rounds. This system of accounting is accomplished by tabulation of the synthesis rounds which are correlated with addition of fragments. While for the purposes of illustration of the invention, a tabulation method of tracking fragment addition is described herein, it will be recognized by the art-skilled that other algorithms, algorithmic codes, computer readable mediums and various software coding techniques know to those skilled in the computer arts may be used for such tracking. The tables tracking fragment addition can be used to produce structural representations of compounds and create virtual libraries where actual synthesis of the compounds is not desired. Tables tracking transformations, however, can be used to synthesize compounds by selecting the appropriate transformations, and in the case of multiple transformations, selecting the preferable transformations to introduce the required fragment into the compounds being synthesized.

Figure 15 is descriptive of compound C1 in terms of the fragments added in each synthesis round. The first synthesis round (*i.e.* round n) commences with the selection of fragment F7. This is followed by the sequential addition of fragments F2, F1 and F5 in the second synthesis round (*i.e.* round n+1). Finally, compound C1 is generated by the addition of fragment F3 in the third synthesis round (*i.e.* round n+2). The compounds thus generated can be stored as a 2-dimensional virtual library, or may be converted to a 3-dimensional virtual library that can be used for *in silico* docking to desired target molecules.

For the generation of virtual libraries of compounds and for docking the library members onto target molecules, it suffices to add compounds to the relational database in terms of its fragments to track the addition of fragments in the various synthetic rounds. However, when the actual synthesis of desired compounds of a library is to be undertaken, it becomes necessary to specify the actual synthetic steps, reagents, solvents, concentrations, auxiliary compounds needed and other various synthetic factors in order to effect such an actual synthesis of real chemical compounds. Such synthetic steps, reagents, solvents, concentrations and auxiliary compounds are, in fact, incorporated in to the above described

transformations. Thus by employing the concept of transformations, the present invention provides methods to track the compounds generated not only in terms of the fragments added but as well as the synthetic parameters necessary for each synthesis round.

Figure 15 also shows the generation of compound C1 in terms of the various transformations employed in the synthesis rounds. Four synthesis pathways lead to the synthesis of compound C1 because of the availability of multiple transformations that can introduce the same fragment into the compound being synthesized. Thus, as seen in Figure 15, selection of fragment F7 constitutes transformation T9 in the first synthesis round (*i.e.* round n). This is followed by the addition of fragment F2 which is achieved by employing transformation T2. Next, fragment F1 is added via transformation T1. Fragment F5, however, may be added by employing either reagent R6 via transformation T6 along synthesis paths 1 and 3, or reagent R7 via transformation T7 along synthesis paths 2 and 4. Similarly, the final fragment F3 can be added by using either reagent R3 via transformation T3 along synthesis paths 1 and 2, or reagent R4 via transformation T4 along synthesis paths 3 and 4. Thus Figure 15 shows that compound C1 can be actually synthesized via one of four different synthetic schemes which can be tracked or tabulated and accounted for using the methods of the present invention. Each of the four tables is completely descriptive of each of the four synthetic pathways for the preparation of C1. Thus, a user of the present invention has available all the alternate pathways of performing the same reaction (*i.e.* introducing the same fragment), and can select the preferable or most appropriate synthetic route to preparing the desired compounds.

Figure 16 shows a similar transformation tracking table for compounds C2 and C3 in mixture M1. Synthesis of compounds C2 and C3 commences with selection of fragment F7 which represents transformation T9 (step 1 in Figure 16) in the first synthesis round (*i.e.* round n). Next, F7 is combined with fragment F2 via transformation T2 in the second synthesis round (*i.e.* round n+1) (step 2). In the same round, fragment F1, via transformation T1, and fragment F5, via transformation T7 are added sequentially (steps 3 and 4). Finally, fragment F4 is added in the third synthesis round (*i.e.* round n+2). As F4 is a mixture of two constituent fragments (because of two constituent reagents), the table is duplicated at this stage (step 5) to account for the different synthetic ways in which transformation T5 may be accomplished (*i.e.* $T5^1$ and $T5^2$). Step 5 represents compounds C2 and C3. Thus it can be seen that, in

accordance with the present invention, whenever there is more than one reagents associated with a particular transformation, the table is duplicated as many times as there are such reagents.

Figure 17 shows a transformation tracking table for compounds C1 and C5 in mixture M3. As the synthesis commences with two fragments, F7 and F8, tracking begins with two parallel tables (step 1 in Figure 17). In the first synthesis round (*i.e.* round n), F7 is selected via transformation T9, while F8 is selected via transformation T10. The second synthesis round (*i.e.* round n+1) commences at step 2 with the introduction of fragment F2 via transformation T2. In step 3, transformation T1 introduces fragment F1 into the compound. In step 4, transformation T7 introduces fragment F5. This completes the second synthesis round (*i.e.* round n+1). Finally, in the third synthesis round (*i.e.* round n+2), transformation T4 is used to introduce fragment F3 (at step 5) producing mixture M2 comprising compounds C1 and C5. In this example, the tables are duplicated early in the synthetic scheme because of the use of a mixture of fragments F7 and F8 at the outset.

The transformation tracking table for compounds C2, C3, C7 and C8 of mixture M3 are shown in Figure 18. The synthesis of these compounds commences with the first synthesis round (*i.e.* round n) in which fragment F7 is selected. This represents transformation T9 (shown in step 1 in Figure 18). Step 2 in Figure 18 depicts the second synthesis round (*i.e.* round n+1) and involves the addition of fragment F2 via transformation T2. While steps 1 and 2 involve single transformations each, step 3 involves two different transformations because two different fragments are being introduced into the compounds through the use of two different reagents. Therefore, at step 3 the table is twice duplicated because two different reagents are being employed to introduce two different fragments via two different transformations. In step 3, transformation T1 is used to introduce fragment F1 while transformation T3 is used to introduce fragment F3. The second synthesis round (*i.e.* round n+1) is completed at step 4 with transformation T7 which introduces fragment F5. In the final synthesis round (*i.e.* the third round or round n+2), transformation T5 is used to introduce fragment F4. As F4 is a mixture of two constituent fragments, each table at step 5 is twice duplicated for transformations $T5^1$ and $T5^2$ which represent each of the constituent fragments of F4.

These figures represent merely one manner in which the various fragments, reagents

and transformations may be tracked during the generation or synthesis of single compounds or mixtures of compounds. It will, however, be recognized by the art-skilled that various other algorithm schemes may be employed to track and account for the fragments being introduced via transformations when compounds are being generated *in silico*.

The libraries as described above as well as libraries created by other means, can be synthesized on various automated synthesizers. For illustrative purposes, the synthesizer utilized for synthesis of above described libraries, is a variation of the synthesizer described in United States patents 5,472,672 and 5,529,756, the entire contents of which are herein incorporated by reference. The synthesizer described in those patents was modified to include movement in along the Y axis in addition to movement along the X axis. As so modified, a 96-well array of compounds can be synthesized by the synthesizer. The synthesizer can further include temperature control and the ability to maintain an inert atmosphere during all phases of a synthesis. The reagent array delivery format employs orthogonal X-axis motion of a matrix of reaction vessels and Y-axis motion of an array of reagents. Each reagent has its own dedicated plumbing system to eliminate the possibility of cross-contamination of reagents and line flushing and/or pipette washing. This in combined with a high delivery speed obtained with a reagent mapping system allows for the extremely rapid delivery of reagents. This further allows long and complex reaction sequences to be performed in an efficient and facile manner.

Software, as described below utilized in conjunction with the synthesizer allows the straightforward programming of the parallel synthesis of a large number of compounds. The software utilizes a general synthetic procedure in the form of a command (.cmd) file, which calls upon certain reagents to be added to certain wells *via* lookup in a sequence (.seq) file. The bottle position, flow rate, and concentration of each reagent is stored in a lookup table (.tab) file. Thus, once a synthetic method is outlined, a plate of compounds is made by permutating a set of reagents, and writing the resulting output to a text file. The text file is input directly into the synthesizer and used for the synthesis of the plate of compounds. The synthesizer can be interfaced with a relational database allowing data output related to the synthesized compounds to be registered in a highly efficient manner.

The .seq, .cmd and .tab files are built or constructed and once constructed, are stored in an appropriate database. The .cmd file is a synthesis file. This file can be built fresh to

reflect a completely new set of machine commands reflecting a set of chemical synthesis steps (as for instance the above described transformations) or it can modify an existing file stored in a database by editing a stored file. The .cmd files are built using a word processor and a command set of instructions as outlined below.

In a like manner to the building the .cmd files, .tab files are built to reflect the necessary reagents used in the automatic synthesizer for the particular chemistries necessary for the library of desired compounds. Thus for each of a set of these chemistries, a .tab file is built and stored in the database. As with the .cmd files, an existing .tab file can be edited for use in constructing a further .tab file.

Both the .cmd files and the .tab files are linked together for later retrieval from the database. Linking can be as simple as using like file names to associate a .cmd file to its appropriate .tab file, e.g., syntheses.cmd is linked to syntheses.tab by use of the same preamble in their names.

The automated, multi-well parallel array synthesizer employs a reagent array delivery format, in which each reagent utilized has a dedicated plumbing system. As seen in Figures 19 and 20, an inert atmosphere **10** is maintained during all phases of a synthesis. Temperature is controlled *via* a thermal transfer plate **12**, which holds an injection molded reaction block **14**. The reaction plate assembly slides in the X-axis direction, while eight nozzle blocks (**16, 18, 20, 22, 24, 26, 28** and **30**) holding the reagent lines slide in the Y-axis direction, allowing for the extremely rapid delivery of any of 64 reagents to 96 wells. In addition, there are six banks of fixed nozzle blocks (**32, 34, 36, 38, 40** and **42**) which deliver the same reagent or solvent to eight wells at once, for a total of 72 possible reagents. In synthesizing compounds for screening, the target reaction vessels, a 96 well plate **44** (a 2-dimensional array), moves in one direction along the X axis, while the series of independently controlled reagent delivery nozzles (**16, 18, 20, 22, 24, 26, 28** and **30**) move along the Y-axis relative to the reaction vessel **46**. As the reaction plate **44** and reagent nozzles (**16, 18, 20, 22, 24, 26, 28** and **30**) can be moved independently at the same time, this arrangement facilitated the extremely rapid delivery of up to 72 reagents independently to each of the 96 reaction vessel wells.

The system software allows the straightforward programming of the synthesis of a large number of compounds by supplying the general synthetic procedure in the form of the command file to call upon certain reagents to be added to specific wells *via* lookup in the

sequence file with the bottle position, flow rate, and concentration of each reagent being stored in the separate reagent table file. Compounds can be synthesized on various scales ranging from small, as for example a 200 nmole scale, to larger scales, as for example a 10 μmole scale (3-5 mg). The resulting crude compounds are generally >80% pure, and are utilized directly for high throughput screening assays. Alternatively, prior to use the plates can be subjected to quality control to ascertain their exact purity. Use of the synthesizer results in a very efficient means for the parallel synthesis of compounds for screening.

The software inputs accept tab delimited text files from any text editor. A typical command file, a .cmd file, is shown in Example 4, Table 2. A typical sequence file, a .seq files, is shown in Example 4, Table 3, and a typical reagent file, a .tab file, is shown in Example 4, Table 4. Typically some of the wells of the 96 well plate may be left empty (depending on the number of compounds in the individual synthesis) or some of the well may have compounds that will serve as standards for comparison or analytical purposes.

Prior to loading reagents, moisture sensitive reagent lines are purged with argon at **10** for 20 minutes. Reagents are dissolved to appropriate concentrations and installed on the synthesizer. Large bottles, collectively identified as **46** in Figure 19 (containing 8 delivery lines) are used for wash solvents and the delivery of general activators, cleaving reagents and other reagents that may be used in multiple wells during any particular synthesis. Small septa bottles, collectively identified as **48** in Figure 19, are utilized to contain individual reagent compounds. This allows for anhydrous preparation and efficient installation of multiple reagents by using needles to pressurize the bottle, and as a delivery path. After all reagents are installed, the lines are primed with reagent, flow rates measured, then entered into the reagent table (.tab file). A dry resin loaded plate is removed from vacuum and installed in the machine for the synthesis.

The modified 96 well polypropylene plate **44** is utilized as the reaction vessel. The working volume in each well is approximately 700 μl. The bottom of each well is provided with a pressed-fit 20 μm polypropylene frit and a long capillary exit into a lower collection chamber as is illustrated in Figure 5 of the above referenced United States Patent 5,372,672. The solid support for use in holding the growing compounds during synthesis is loaded into the wells of the synthesis plate **44** by pipetting the desired volume of a balanced density slurry of the support suspended in an appropriate solvent, typically an acetonitrile-methylene

chloride mixture. Reactions can be run on various scales as for instance the above noted 200 nmole and 10 μmol scales. Various supports can be utilized for synthesis. Particularly useful supports include medium loading polystyrene-PEG supports such as TentaGel™ or ArgoGel™.

As seen in Figure 20, the synthesis plate is transported back and forth in the X-direction under an array of 8 moveable banks (**16, 18, 20, 22, 24, 26, 28** and **30**) of 8 nozzles (64 total) in the Y-direction, and 6 banks (**32, 34, 36, 38, 40** and **42**) of 48 fixed nozzles, so that each well can receive the appropriate amounts of reagents and/or solvents from any reservoir (large bottle or smaller septa bottle). A sliding balloon-type seal **50** surrounds this nozzle array and joins it to the reaction plate headspace **52**. A slow sweep of nitrogen or argon **20** at ambient pressure across the plate headspace is used to preserve an anhydrous environment.

The liquid contents in each well do not drip out until the headspace pressure exceeds the capillary forces on the liquid in the exit nozzle. A slight positive pressure in the lower collection chamber can be added to eliminate residual slow leakage from filled wells, or to effect agitation by bubbling inert gas through the suspension. In order to empty the wells, the headspace gas outlet valve is closed and the internal pressure raised to about 2 psi. Normally, liquid contents are blown directly to waste **54**. However, a 96 well microtiter plate can be inserted into the lower chamber beneath the synthesis plate in order to collect the individual well eluent for spectrophotometric monitoring of reaction progress and yield.

The basic plumbing scheme for the machine is the gas-pressurized delivery of reagents. Each reagent is delivered to the synthesis plate through a dedicated supply line, collectively identified at **56**, solenoid valve collectively identified at **58** and nozzle, collectively identified at **60**. Reagents never cross paths until they reach the reaction well. Thus, no line needs to be washed or flushed prior to its next use and there is no possibility of cross-contamination of reagents. The liquid delivery velocity is sufficiently energetic to thoroughly mix the contents within a well to form a homogeneous solution, even when employing solutions having drastically different densities. With this mixing, once reactants are in homogeneous solution, diffusion carries the individual components into and out of the solid support matrix where the desired reaction takes place. Each reagent reservoir can be plumbed to either a single nozzle or any combination of up to 8 nozzles. Each nozzle is also

provided with a concentric nozzle washer to wash the outside of the delivery nozzles in order to eliminate problems of crystallized reactant buildup due to slow evaporation of solvent at the tips of the nozzles. The nozzles and supply lines can be primed into a set of dummy wells directly to waste at any time.

The entire plumbing system is fabricated with Teflon tubing, and reagent reservoirs are accessed *via* syringe needle/septa or direct connection into the higher capacity bottles. The septum vials **48** are held in removable 8-bottle racks to facilitate easy setup and cleaning. The priming volume for each line is about 350 μl. The minimum delivery volume is about 2 μl, and flow rate accuracy is ±5%. The actual amount of material delivered depends on a timed flow of liquid. The flow rate for a particular solvent will depend on its viscosity and wetting characteristics of the Teflon tubing. The flow rate (typically 200-350 μl per sec) is experimentally determined, and this information is contained in the reagent table setup file.

Heating and cooling of the reaction block **14** is effected utilizing a recirculating heat exchanger plate **12**, similar to that found in PCR thermocyclers, that nests with the polypropylene synthesis plate **44** to provide good thermal contact. The liquid contents in a well can be heated or cooled at about 10°C per minute over a range of +5 to +80°C, as polypropylene begins to soften and deform at about 80°C. For temperatures greater than this, a non-disposable synthesis plate machined from stainless steel or monel with replaceable frits might be utilized.

The hardware controller is designed around a set of three 1 MHZ 86332 chips. This controller is used to drive the single X-axis and 8 Y-axis stepper motors as well as provide the timing functions for a total of 154 solenoid valves. Each chip has 16 bidirectional timer I/O and 8 interrupt channels in its timer processing unit (TPU). These are used to provide the step and direction signals, and to read 3 encoder inputs and 2 limit switches for controlling up to three motors per chip. Each 86332 chip also drives a serial chain of 8 UNC5891A darlington array chips to provide power to 64 valves with msec resolution. The controller communicates with the Windows software interface program running on a PC via a 19200 Hz serial channel, and uses an elementary instruction set to communicate valve_number and time_open, and motor_number and position_data.

The three components of the software program that run the array synthesizer, the generalized procedure or command (.cmd) file which specifies the synthesis instructions to

be performed, the sequence (.seq) file which specifies the scale of the reaction and the order in which variable groups will be added to the core synthon, and the reagent table (.tab) file which specifies the name of a chemical, its location (bottle number), flow rate, and concentration are utilized in conjunction with a basic set of command instructions.

The basic set of command instructions are:

ADD

IF {block of instructions} END_IF

REPEAT {block of instructions} END_REPEAT

PRIME, NOZZLE_WASH

WAIT, DRAIN

LOAD, REMOVE

NEXT_SEQUENCE

LOOP_BEGIN, LOOP_END

The ADD instruction has two forms, and is intended to have the look and feel of a standard chemical equation. Reagents are specified to be added by a molar amount if the number proceeds the name identifier, or by an absolute volume in micro liters if the number follows the identifier. The number of reagents to be added is a parsed list, separated by the '+' sign. For variable reagent identifiers, the key word, <seq>, means look in the sequence table for the identity of the reagent to be added, while the key word, <act>, means add the reagent which is associated with that particular <seq>. Reagents are delivered in the order specified in the list.

Thus:

ADD ACN 300

means: Add 300 μl of the named reagent ACN to each well of active synthesis

ADD <seq> 300

means: If the sequence pointer in the .seq file is to a reagent in the list of reagents, independent of scale, add 300 μl of that particular reagent specified for that well.

ADD 1.1 PYR + 1.0 <seq> + 1.1 <act1>

means: If the sequence pointer in the .seq file is to a reagent in the list of acids in the Class ACIDS_1, and PYR is the name of pyridine, and ethyl

chloroformate is defined in the .tab file to activate the class, ACIDS_1, then this instruction means:

Add 1.1 equiv. pyridine
1.0 equiv. of the acid specified for that well and
1.1 equiv. of the activator, ethyl chloroformate

The IF command allows one to test what type of reagent is specified in the <seq> variable and process the succeeding block of commands accordingly.

Thus:

```
ACYLATION                {the procedure name}
    BEGIN
        IF CLASS = ACIDS_1
            ADD 1.0 <seq> + 1.1 <act1> + 1.1 PYR
            WAIT 60
        ENDIF
        IF CLASS = ACIDS_2
            ADD 1.0 <seq> + 1.2 <act1> + 1.2 TEA
        ENDIF
        WAIT 60
        DRAIN 10
    END
```

means: Operate on those wells for which reagents contained in the Acid_1 class are specified, WAIT 60 sec, then operate on those wells for which reagents contained in the Acid_2 class are specified, then WAIT 60 sec longer, then DRAIN the whole plate. Note that the Acid_1 group has reacted for a total of 120 sec, while the Acid_2 group has reacted for only 60 sec.

The REPEAT command is a simple way to execute the same block of commands multiple times.

Thus:

```
WASH_1              {the procedure name}
    BEGIN
        REPEAT 3
            ADD ACN 300
```

DRAIN 15

END_REPEAT

END

means: repeats the add acetonitrile and drain sequence for each well three times.

The PRIME command will operate either on specific named reagents or on nozzles which will be used in the next associated <seq> operation. The μl amount dispensed into a prime port is a constant that can be specified in a config.dat file.

The NOZZLE_WASH command for washing the outside of reaction nozzles free from residue due to evaporation of reagent solvent will operate either on specific named reagents or on nozzles which have been used in the preceding associated <seq> operation. The machine is plumbed such that if any nozzle in a block has been used, all the nozzles in that block will be washed into the prime port.

The WAIT and DRAIN commands are by seconds, with the drain command applying a gas pressure over the top surface of the plate in order to drain the wells.

The LOAD and REMOVE commands are instructions for the machine to pause for operator action.

The NEXT_SEQUENCE command increments the sequence pointer to the next group of substituents to be added in the sequence file. The general form of a .seq file entry is the definition:

Well_No Well_ID Scale Sequence

The sequence information is conveyed by a series of columns, each of which represents a variable reagent to be added at a particular position. The scale (μmole) variable is included so that reactions of different scale can be run at the same time if desired. The reagents are defined in a lookup table (the .tab file), which specifies the name of the reagent as referred to in the sequence and command files, its location (bottle number), flow rate, and concentration. This information is then used by the controller software and hardware to determine both the appropriate slider motion to position the plate and slider arms for delivery of a specific reagent, as well as the specific valve and time required to deliver the appropriate reagents. The adept classification of reagents allows the use of conditional IF loops from

within a command file to perform addition of different reagents differently during a 'single step' performed across 96 wells simultaneously. Reagents can be group according to "class." Thus all for a particular synthesis that utilizes a fragment that is based on amino acids, the class "AMINO_ACIDS" can be created. The special class ACTIVATORS defines certain reagents that always get added with a particular class of reagents (for example Betaine utilized to activate the class AMINO_ACIDS).

The general form of the .tab file is the definition:

Class	Bottle	Reagent Name	Flow_rate	Conc.

The LOOP_BEGIN and LOOP_END commands define the block of commands which will continue to operate until a NEXT_SEQUENCE command points past the end of the longest list of reactants in any well.

Not included in the command set is a MOVE command. For all of the above commands, if any plate or nozzle movement is required, this is automatically executed in order to perform the desired solvent or reagent delivery operation. This is accomplished by the controller software and hardware, which determines the correct nozzle(s) and well(s) required for a particular reagent addition, then synchronizes the position of the requisite nozzle and well prior to adding the reagent.

A MANUAL mode is also utilized in which the synthesis plate and nozzle blocks can be 'homed' or moved to any position by the operator, the nozzles primed or washed, the various reagent bottles depressurized or washed with solvent, the chamber pressurized, etc. The automatic COMMAND mode can be interrupted at any point, MANUAL commands executed, and then operation resumed at the appropriate location. The sequence pointer can be incremented to restart a synthesis anywhere within a command file.

The compounds to be synthesized can be rearranged or grouped for optimization of synthesis. Such grouping can be effected based on any parameter that will result in optimization of synthesis. One such factor considers the fragment of the compounds that are directly linked to the supporting resin. If the same fragment is to be utilized multiple times, it can be joined to the support in a batch wise manner and aliquots of this batch synthesis then loaded into the individual wells of the plate prior to start of the synthesis. Another parameter

is by positioning like compounds near each other. By grouping like fragments near each other, machine movements are conserved and in doing so, overall synthesis time is shortened.

In utilizing the multi well format for compound synthesis, for each compound to be synthesized, an aliquot of a solid support bearing the proper first fragment thereon can be added to the well for synthesis. Thus prior to loading the sequence of compounds to be synthesized in the .seq file, they are sorted by this fragment. Based on that sorting, all of compounds having similar first fragments are positioned together in adjacent wells on the plate. Thus in loading the fragment-bearing solid support into the synthesis wells, machine movements are conserved. In a further method of preparing compounds, only the solid support is added to the wells and the first fragment is then linked to the solid support as the first synthetic step. The .seq file is appropriately modified to reflect that the first segment is to be added.

Once sorted into types, the position of the compounds on the synthesis plates is specified by the creation of a .seq file as described above. The .seq file is associated with the respective .cmd and .tab files needed for synthesis of the particular chemistries specified for the compounds by retrieval of the .cmd and .tab files a database. These files are then input into the multi well synthesizer for compound synthesis. Upon completion of synthesis, for shipping, storage or other handling purposes, the plates can be lyophilized at this point if desired. Upon lyophilization, each well contains the compounds located therein as a dry compound.

To illustrate the invention, a synthetic was effected utilizing the methods of the invention to generate a small library (~1200) of discreet hydroxamic acids. The total library is shown in Table 1 below. Two distinct chemical pathways were utilized for the automated synthesis of the illustrative library of hydroxamic acid compounds. These are shown in Figures 21 and 22. Each pathway had its own advantages.

The illustrative hydroxamic library compounds generally correspond in structure to compound CI of Figure 1, formed from a hydroxylamine fragment, a valine fragment (the amino acid fragment) and a sulfonyl-4-methoxybenzene fragment (the sulfonyl fragment) of Figure 2. They differ from one another with respect to their amino acid fragment and their sulfonyl fragment. They have in common their hydroxyl amine fragment. Compound CI directly corresponds (they are one in the same) to compound **a-x** of Table 1. These

compounds further corresponds to symbolic compound CI'.

For illustrative purposes to demonstrate complex chemical structures and mixtures, the symbolic tables shown in the Figures 15, 16, 17 and 18 described certain complex symbolic structures and thus equally complex chemical structures. Compared to these complex structures and mixtures, compound CI' is less complex, however, its construction embodies the same principles as used to describe the structures of those figures. Since it embodies the same principles, one can construct a similar table for compound CI'. Thus in round n it would have the fragment Fi', in round n+1 the fragment Fii' and in round n+2, the fragment Fiii'. A transformation table can likewise be constructed listing Ti in round n, Tii in round n+1 and Tiii in round n+2. This information is then used to instruct the automated synthesizer to prepare the actual library.

In constructing the illustrative hydroxamic library utilizing the synthetic pathway of Figure 22, the first fragment, the hydroxyl amine fragment is the same in all members of the library. Therefore, for ease of synthesis, it is added already attached to a solid support to wells in a synthesis plate. This reduces the complexity of the synthesis by a factor of "one fragment" and in turn reduce the number of rounds by one of synthesis that must be effected on the synthesizer. In essence this eliminates the round n as described in the tables of Figures 15, 16, 17 and 18.

As described above, the general form of a .seq file entry was:

Well_No	Well_ID	Scale	Sequence

where the "Sequence" information was conveyed by a series of columns. Since the round n transformation has been generalized for each well on the plate by adding the hydroxyl amine fragment attached to a solid support, only two Sequence columns are necessary to describe the synthesis, one for the round n+1 showing the amino acid reagent used and one for the round n+2 showing the sulfonyl reagent used. Each "Sequence" column corresponds to a reagent which is a member of a transformation represented in the tracking tables. This reagent is linked by the one to one relationship specified by the transformation to its resulting fragment.

Various algorithms, as will be evident to those skilled in the computer programing

arts, could be utilized to convert the information contained withing the tracking tables described above into the format suitable for synthesis utilizing the parallel array synthesizer described herein. One preferred way to accomplish this is by looking up the transformation required for each particular round of synthesis for each compound or group of compounds in the tracking table. The appropriate complex or single reagent is then written to a software file in a format such that each reagent utilized for the transformation indicated in the tracking table at the appropriate synthesis round corresponds to a single column entry in the .seq file. The compounds or groups of compounds stored in the database and their location in the reaction vessel in which they are synthesized are then linked by the Well_ID field of the .seq file, which is assigned by the database. Thus, having described the compounds by their transformations allows for facile construction of the .seq file need for synthesis. This is illustrated by the synthesis files for the parallel array synthesizer detailed above, but the process is equally applicable to any suitable programmable chemical synthesis apparatus.

In a like manner the general form of the .tab file was:

Class	**Bottle**	**Reagent Name**	**Flow Rate**	**Conc.**

Here complex or single reagents can be specified in the "Reagent Name" as defined by the bottle the reagent or mixture of reagents is located. Whether it was a single reagent or a complex reagent mixture specified by a particular transformation, that information is carried over to the synthesizer instructions by the appropriate entry in the .tab file for that reagent. As for the .seq file creation, the information in the transformation tracking table can be readily converted to a .tab file. Each complex or single reagent called for in the synthesis is given a line entry in the .tab file. Additionally, the single reagent components of complex reagents may be specified in a comments section of the .tab file to facilitate preparation of complex reagents. The appropriate conditions for the specified reagent as indicated in the corresponding transformation are also written to the proscribed field in the .tab file. Additionally, associated reagents for accomplishing the specified transformation (such as activators, bases, scavangers, coupling reagents, etc.) may also be written to the .tab file as appropriate. In the synthesis of the illustrative hydroxamic acid library, the activator named "betaine" is associated with the transformation attaching the amino acid to solid support. It

is placed in the .tab file, along with a modifier specifying which reagents it is associated. As a result of having described the compounds by their transformations, construction of the .tab file need for synthesis is facilitate. This is illustrated by the synthesis files for the parallel array synthesizer detailed herein, but the process is equally applicable to any suitable programmable chemical synthesis apparatus.

As the complexity of the fragments for the compounds in a library increases as for instances steps P1a, P1b, P1c, P1c and P3b of Figure 11, they in turn require more column entries the "Sequence" portion of .seq. However, if complexity is achieved by using mixtures of reagents that are used in unison, as for instances step P2 of Figure 11, this is controlled by locating them in a single reagent bottle as specified by the .tab file.

In reference again to the illustrative hydoxamic acid library of Table 1, the first method of synthesis, illustrated in Figure 21, entails derivatizing commercially available ArgoGel-OH™ (which has an PEG based alcohol as the reactive functional group) with an FMOC-amino acid *via* a modified Mitsunobu reaction employing the sulfonamide betaine **1** as the activating species. This reaction proceeded to essentially 100% completion (by FMOC) in several hours, and has the advantage over other loading procedure (symmetric anhydride/DMAP) of eliminating the potential for racemization of the amino acid. It also requires less equivalents, as one equivalent of amino acid is not wasted due to the formation of a symmetric anhydride, and the potential for FMOC loss is minimized. The resin bound ester **2** was next deprotected, then sulfonylated using a sulfonyl chloride in pyridine. The yield of the Mitsunobu loading step was measured by collecting the washes from the FMOC deprotection, followed by spectrophotometric determination of the amount released in a 96 well plate reader. This information was then written to a data file for import into a database, which allows a yield estimate of the synthesized compounds. It was found that cleavage of the ester **4** with hydroxylamine in 1,4-dioxane (50% aqueous NH_2OH diluted to 4 M final NH_2OH concentration with 1,4-dioxane) generally proceeded to completion overnight at room temperature to provide the desired hydroxamic acids **5**. A small amount (10-20%) of the corresponding carboxylic acid resulted from competitive hydrolysis for hindered amino acids such as valine, even when anhydrous hydroxylamine was employed. Several hindered amino acids and electron deficient sulfonyl chlorides failed completely with this method as indicated in Table **1** below.

The procedure has the advantage that orthogonal deprotection and cleavage strategies can be employed, allowing standard peptide acid labile side chain protection (*t*-butyl based, trityl, PMC, etc.) to be used on the amino acid component. This allows isolation of products free from side chain protection by-products in the case of commonly used trityl and sulfonyl based protection of histidine, arginine, glutamine, and asparagine. Thus, the resin bound ester **4** can be treated with anhydrous TFA for 4 h on the instrument, resulting in complete side chain deprotection. If cleaned of TFA immediately after synthesis, the instrument, including lines and valves were unaffected by the extreme conditions. The support could then be washed and the product **5** cleaved from support using the standard methodology. This synthesis was accomplished very readily on the automated parallel array synthesizer, using a very simple command file, which functions as a 'general procedure'. Representative command, sequence and tab files are detailed in the Example 4 below to illustrate the synthesis.

The second method utilized the acid labile Wang based hydroxylamine support **6** (Figure 22) to circumvent the minor problem of competitive hydrolysis, and the failure of electron deficient sulfonyl chlorides. The resin was prepared in an analogous manner to the procedure described by Atheron et al., *Solid Peptide Synthesis: A Practical Approach*; IRL Press: Oxford, UK 1989: p 135 employing an initial Mitsunobu reaction of ArgoGel-Wang™ resin with *N*-hydroxyphthalimide, followed by deprotection with methylhydrazine to afford **6** in quantitative yield by gel-phase ^{13}C NMR. The hydroxylamine resin was then acylated with an FMOC-amino acid utilizing standard peptide coupling methodology to provide **7**, which was deprotected then sulfonylated as before to provide resin bound hydroxamic acid **8**. This material was efficiently cleaved from the resin with TFA containing Et_3SiH (5% v/v) as a scavenger to provide compounds **5**.

EXAMPLES

Example 1: General Procedure for Automated Synthesis of Library Plates

ArgoGel-OH™ (360 mg, loading 0.43 mmole/g) was suspended in ~16 mL solution of 3:1 CH_2Cl_2/DMF. The suspension was distributed equally among 12 wells of a 96 well polypropylene synthesis plate (30 mg per well). The solvent was drained and the resin dried overnight in vacuo over P_2O_5. All solid reagents were dried in vacuo overnight over P_2O_5

prior to use. For method 1, the Mitsunobu reagent **1** was dried, then dissolved in anhydrous CH_2Cl_2 to a concentration of 0.15M. FMOC-Amino Acids (Novabiochem, Bachem CA) were dissolved to a concentration of 0.30 M in a solution of 2:1 anhydrous CH_2Cl_2/DMF for method 1, and to a concentration of 0.22 M in DMF containing 0.44 M collidine for synthesis for method 2. Sulfonyl chlorides were dissolved to a concentration of 0.2M in Pyridine. Pyridine proved to be an acceptable solvent for most sulfonyl chlorides, but when solubility was limited, cosolvents such as MeCN, DMSO, CH_2Cl_2, DMF, and NMP (up to 50%) have been employed. FMOC protection were removed with a solution of 10% piperidine in anhydrous DMF prepared and used the day of synthesis. Low water wash solvents were employed to ensure maximum coupling efficiency of the initial amino-acid to the resin. Prior to loading reagents, moisture sensitive reagent lines were purged with argon for 20 minutes. Reagents were dissolved to appropriate concentrations and installed on the synthesizer. Large bottles (containing 8 delivery lines) were used for wash solvents and the delivery of activator. Small septa bottles containing the amino acids and sulfonyl chlorides allow anhydrous preparation and efficient installation of multiple reagents by using needles to pressurize the bottle, and as a delivery path. After all reagents were installed, the lines were primed with reagent, flow rates measured, then entered into the reagent table (.tab file) and the dry resin loaded plate removed from vacuum and installed in the machine for subsequent synthesis. After cleavage from support and centrifugal evaporation of solvent, the products were dissolved in MeOH/CH_2Cl_2 mixtures, then assayed for purity by TLC (typically 10% MeOH/CH_2Cl_2) on silica gel using both UV and I_2 visualization, and for product identity by electrospray mass spectroscopy (negative mode). Selected samples were dissolved in DMSO-d_6 and examined by ^{1}H NMR.

Example 2: General Hydroxamic Acid Synthesis Method 1 (Figure 21)

The commercial ArgoGel-OH™ resin (10 μmole) was washed with CH_2Cl_2 (6x), then treated with the appropriate FMOC-amino acid (3 eq.) and **1** (3 eq.). After 30 min, the wells were drained, and the process repeated to give a total of 4 treatments (12 eq.). The resin was washed with CH_2Cl_2 (6x), DMF (4x), and the FMOC removed with 10% piperidine in DMF (4 x). The washes were collected, diluted appropriately, and the amount of FMOC chromophore released quantitated by UV (ϵ 7800 L*mol^{-1}*cm^{-1}, λ=301 nm). This value was

used to calculate the yield of the final products. The resin was then washed with DMF (4x), then CH_2Cl_2 (6x), and treated with the appropriate sulfonyl chloride (4 x 6 eq. for 15 min.) in pyridine, and washed with CH_2Cl_2 (6x), DMF (6x), and CH_2Cl_2 (10x). At this point the resin could be treated with 90:5:5 TFA/H_2O/Et_3SiH for 4 h, then subjected to the above washing procedure to remove any side chain protection on the molecules if necessary. The plates were then removed from the instrument, and individual wells treated with 4 M hydroxylamine (50% aqueous) in 1,4-dioxane for 24 h. The filtrate was collected into a deep well 96 well plate, the samples frozen, then lyophilized to provide the desired hydroxamic acids. Addition of fresh 1,4-dioxane and repetition of the lyophilization process twice gave compounds free of any residual hydroxylamine (by ^1H NMR of selected products).

Example 3: General Hydroxamic Acid Synthesis Method 2 (Figure 22)

Resin **6** was prepared from ArgoGel-Wang-OH™ resin according to published procedures and this resin (10 μmole) was washed with DMF (6x), CH_2Cl_2 (6x), then treated with the appropriate FMOC-amino acid (3 eq.) in DMF + collidine (6 eq.) and HATU (3 eq.). After 30 min, the wells were drained, and the process repeated to give a total of 4 treatments (12 eq.). The resin was washed with CH_2Cl_2 (6x), DMF (4x), and the FMOC removed with 10% piperidine in DMF (4 x). The washes were collected, diluted appropriately, and the amount of FMOC chromophore released quantitated by UV (ϵ 7800 $L*mol^{-1}*cm^{-1}$, λ=301 nm). This value was used to calculate the yield of the final products. The resin was washed with DMF (4x), then CH_2Cl_2 (6x), and treated with the appropriate sulfonyl chloride (4 x 6 eq. for 15 min.) in pyridine, and washed with CH_2Cl_2 (6x), DMF (8x), DMSO (8x), and CH_2Cl_2 (10x). The plates were then removed from the instrument, and individual wells treated with 90:5:5 TFA/Et_3SiH/H_2O for 4 h. The filtrate was collected into a deep well 96 well plate, the resin washed (3x) with TFA, and the samples concentrated in a centrifugal vacuum concentrator. Addition of fresh 1,4-dioxane or isopropanol and repetition of the concentration process twice, followed by drying *in vacuo* overnight gave the desired hydroxamic acids.

The methods of both Examples 1 and 2 were utilized to produce a library of compounds resulting from the combination of FMOC-amino acids and sulfonyl chlorides shown in Table 1.

Table 1. Reagents Used to Prepare Hydroxamic Acids 5 by Automated Synthesis[a]

FMOC-Amino Acid Used[b]		Sulfonyl Chloride Used[c]	
a	D-Val[d]	**i**	1-napthalene
b	D-Ile	**ii**	2-napthalene
c	D-Leu	**iii**	2-thiophene
d	D-Ala	**iv**	2-mesitylene
e	D-cyclo-hexyl-Ala	**v**	3-nitrobenzene
f	D-norvaline	**vi**	4-bromobenzene
g	D-norleucine	**vii**	4-chlorobenzene
h	D-alloiso-leucine	**viii**	4-iodobenzene
i	D-α-t-Butylglycine[e]	**ix**	4-nitrobenzene
j	D-Met	**x**	4-methoxybenzene[d]
k	D-Phenyl-glycine	**xi**	4-t-Butylbenzene
l	D-Phe	**xii**	trifluoromethane[d]
m	D-4-Chloro-Phe	**xiii**	-toluene
n	3-(2-napthyl)- D-Ala	**xiv**	3-(trifluoromethyl)benzene
o	3-(3-pyridyl)-D-Ala	**xv**	4-(trifluromethoxy)benzene
p	-(2-thienyl)-D-Ala	**xvi**	4-(methylsulfonyl)benzene
q	D-Tyr(tBu)[d]	**xvii**	4-(benzenesulfonyl)thiophene-2-
r	D-Trp	**xviii**	4-ethylbenzene
s	D-Cys(tBu)	**xix**	4-cyanobenzene
t	S-Bn-D-penicillamine	**xx**	4-methoxy-2,3,6-trimethylbenzene
u	glycine	**xxi**	benzo-2,1,3-thiadiazole-4-
v	aminoisobutyric acid	**xxii**	1-Methylimidazole-4-
w	D-Thr(tBu)[e]	**xxiii**	5-chloro-3-methylbenzo[B] thiophene-2-[d]
x	D-Ser(tBu)	**xxiv**	benzofurazan-4-
y	D-His(Trt)[d]	**xxv**	3,5-dichlorobenzene
z	D-Pro	**xxvi**	3,4-dimethoxybenzene
aa	D-Tic	**xxvii**	4-(n-butoxy)benzene
bb	D-Lys(BOC)	**xxviii**	2,4-dichlorobenzene
cc	D-Asp(OtBu)	**xxix**	4-trifluoromethylbenzene
dd	D-Glu(OtBu)	**xxx**	2,5-dimethoxybenzene
ee	L-Val	**xxxi**	3,4-dichlorobenzene[d]
ff	L-Ala	**xxxii**	4-n-propylbenzene[d]
gg	L-Phe[d]	**xxxiii**	4-isopropylbenzene[d]
hh	D-Asn(Trt)[e]	**xxxiiv**	2,5-dichlorothiophene-3-
ii	D-Gln(Trt)[e]	**xxxv**	2-[1-methyl-5-(trifluoromethyl) pyrazol-3-yl]thiophene-5-
jj	D-Arg(Pmc)[d]	**xxxvi**	2-[3-(trifluoromethyl)pyrid-2-yl sulfonyl]thiophene-5-

[a]All possible combinations of reagents shown were utilized to attempt the preparation of 1296 hydroxamic acids according to method 2 (Figure 22. [b]Standard abbreviations used for FMOC-amino acids. All amino acids used were obtained from Novabiochem, Bachem, or

Synthetech. [c]Truncated chemical names are given in the table. Appending 'sulfonyl chloride' to the prefix listed gives the appropriate name. All sulfonyl chlorides used were obtained from Aldrich, Lancaster, or Maybridge. [d]Also prepared *via* method 1 (Figure **21**). [e]Failed in method 1.

Example 4: Representative parallel array synthesizer input files

The software inputs accept tab delimited text files from any text editor. Examples for the synthesis of hydroxamic acids *via* the procedure of Figure 21 are shown in Table 2 (.cmd file), Table 3 (.seq file), and Table 4 (.tab file). Only several wells worth of synthesis are shown for brevity. For an entire plate to be prepared, only additional sulfonyl chlorides and additional amino acids need to be added to the .tab file, and additional combinations of the two need to be added to the .seq file such that it contains 96 lines, with each line corresponding to a unique compound prepared.

The identity and purity of the compounds was determined by electrospray mass spectroscopy (negative mode) and thin layer chromatography on silica employing $MeOH/CH_2Cl_2$ solvent mixtures (TLC). The synthesis products in approximately every third well were assayed by TLC and electrospray mass spectroscopy, and the desired compounds were generally present with purities of 60 to 90% when using either of the synthesis methods described above.

Table 2. Example .cmd file (general synthesis procedure) which executes the synthesis shown in Figure 21. The cleavage from support with hydroxylamine is performed separately.

```
INITIAL_WASH
    BEGIN
        Repeat 6
            Add CH2Cl2 300
            Drain 20
        End_Repeat
    END
COUPLE_AMINO_ACID
```

```
        BEGIN
            Repeat 4
                Add <SEQ> 100 + <ACT1> 200
                Wait 1800
                Drain 20
            End_Repeat
            Repeat 6
                Add CH2Cl2 300
                Drain 20
            End_Repeat
            Repeat 4
                Add DMF 300
                Drain 20
            End_Repeat
        END
    REMOVE_FMOC
        BEGIN
            Load_Tray
            Repeat 4
                Add PIPERIDINE_DMF 300
                Wait 250
                Drain 20
            End_Repeat
            Remove_Tray
            Repeat 4
                Add DMF 300
                Drain 20
            End_Repeat
            Repeat 6
                Add CH2Cl2 300
                Drain 20
```

```
            End_Repeat
        END
    SULFONYLATE_AMINO_ACID
        BEGIN
            Next_Sequence
            Repeat 4
                Add <SEQ> 300
                Wait 900
                Drain 20
            End_Repeat
            Repeat 6
                Add CH2Cl2 300
                Drain 20
            End_Repeat
        END
    FINAL_WASH
        BEGIN
            Repeat 6
                Add DMF 300
                Drain 20
            End_Repeat
            Repeat 8
                Add CH2Cl2 300
                Drain 20
            End_Repeat
            Repeat 2
                Add CH2Cl2 300
                Drain 60
            End_Repeat
        END
```

Table 3. Example .seq File (list of compounds to make)

1	A1	10	FMOC_D_ALA	4_MEO_BENZENE_SO2CL
2	A2	10	FMOC_D_VAL	2_NAPTHYLENE_SO2CL
3	A3	10	FMOC_D_PHE	3_CF3_BENZENE_SO2CL
4	A4	10	FMOC_D_NAL	4_CL_BENZENE_SO2CL
5	A5	10	FMOC_D_SER(OTBU)	4_MEO_BENZENE_SO2CL
6	A6	10	FMOC_D_ARG_PMC	2_NAPTHYLENE_SO2CL
7	A7	10	FMOC_D_ALA	3_CF3_BENZENE_SO2CL
8	A8	10	FMOC_D_VAL	4_CL_BENZENE_SO2CL
9	A9	10	FMOC_D_PHE	4_MEO_BENZENE_SO2CL
10	A10	10	FMOC_D_NAL	2_NAPTHYLENE_SO2CL
11	A11	10	FMOC_D_SER(OTBU)	3_CF3_BENZENE_SO2CL
12	A12	10	FMOC_D_ARG_PMC	4_CL_BENZENE_SO2CL

Table 4. Example .tab (list of reagents to use)

AMINO_ACIDS

BEGIN

1	FMOC_D_ALA	265	0.30
2	FMOC_D_VAL	265	0.30
3	FMOC_D_PHE	265	0.30
4	FMOC_D_NAL	265	0.30
5	FMOC_D_SER(OTBU)	265	0.30
6	FMOC_D_ARG_PMC	265	0.30

END

SOLVENTS

BEGIN

67	CH2CL2	330	1
66	DMF	240	1

END

SULFONYLCHLORIDES

BEGIN

9	4_MEO_BENZENE_SO2CL	220	0.20
10	2_NAPTHYLENE_SO2CL	220	0.20
11	3_CF3_BENZENE_SO2CL	220	0.20
12	4_CL_BENZENE_SO2CL	220	0.20

END

DEBLOCK

BEGIN

68 PIPERIDINE_DMF 230 1
END

ACTIVATORS
BEGIN
69 BETAINE 300 0.15 Activates AMINO_ACIDS
END

Example 5: Manual solution synthesis of active compounds:

Methyl (2R)-2-amino-3-(2-naphthyl)propanoate.

To a suspension of D-napthylalanine hydrochloride (2.15 g, 10 mmole, Bachem CA) in MeOH (17 mL) was added TMS-Cl (2.8 mL, 22 mmole) dropwise with stirring. The mixture was allowed to stir overnight, and the resulting solution concentrated *in vacuo*, then dried over KOH to afford 2.65 g (100%) of methyl (2*R*)-2-amino-3-(2-naphthyl)propanoate, which was >95% pure by ^{1}H NMR, and used without further purification: R_f 0.63 (4:1:1 *n*-BuOH/AcOH/H_2O); ^{1}H NMR (DMSO-d_6) δ 8.76 (bs, 3H), 8.00-7.30 (m, 7H), 4.39 (t, 1H), 3.69 (s, 3H), 3.66 (m, 2H); MS (APCI$^+$) *m/e* 230 (M+H).

(2R)-2-(((4-bromophenyl)sulfonyl)amino)-3-(2-naphthyl)propanehydroxamic acid (5-n-vi).

A suspension of D-Napthylalanine hydrochloride methyl ester (1.33 g, 5 mmole), (*i*-Pr_2)NEt (2.61 mL, 15 mmole) and 4-bromobenzesulfonyl chloride (1.53 g, 6 mmol) in CH_2Cl_2 (50 mL) was stirred at rt overnight. The solution was washed with 5% $NaHCO_3$, dried (Na_2SO_4), concentrated, then chromatographed (CH_2Cl_2 to 1% MeOH/CH_2Cl_2) and concentrated to provide 2.05 g of the sulfonamide ester. This material was dissolved in 1,4-dioxane (50 mL) and 25 mL of aqueous hydroxylamine (50% w/w) was added. The mixture was allowed to stand at rt for 48 h, then concentrated onto silica, chromatographed (2 % to 10% MeOH/CH_2Cl_2), the solid residue triturated with water, and dried to provide 1.45 g (64%) of **5-n-vi**: R_f 0.35 (2% MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 9.26 (bs, 1H), 7.90-7.20 (m, 11H), 3.88 (dd, 1H), 2.90 (m, 2H) ; MS (electrospray$^-$) *m/e* 447, 449 (M-H). Anal. Calcd for $C_{19}H_{17}N_2O_4SBr$•0.5 H_2O: C, 49.79; H, 3.96; N, 6.11. Found: C, 49.71; H, 3.90; N, 5.97.

(2R)-3-(2-naphthyl)-2-((2-naphthylsulfonyl)amino)propanehydroxamic acid (5-n-ii).

A suspension of D-Napthylalanine hydrochloride methyl ester (1.33 g, 5 mmole), (*i*-Pr_2)NEt (2.61 mL, 15 mmole) and 4-napthalenesulfonyl chloride (1.36 g, 6 mmol) in CH_2Cl_2

(50 mL) was stirred at rt overnight. The solution was washed with 5% $NaHCO_3$, dried (Na_2SO_4), concentrated, then chromatographed (CH_2Cl_2 to 1% MeOH/CH_2Cl_2) and concentrated to provide 2.02 g of the sulfonamide ester. This material was dissolved in 1,4-dioxane (50 mL) and 25 mL of aqueous hydroxylamine (50% w/w) was added. The mixture was allowed to stand at rt for 48 h, then concentrated onto silica, chromatographed (2 % to 10% MeOH/CH_2Cl_2), and dried to provide 1.15 g (55%) of **5-n-ii**: R_f 0.33 (2% MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 9.19 (bs, 2H), 8.17 (s, 1H), 7.95-7.35 (m, 12H), 7.17 (d, 1H), 3.97 (t, 1H), 2.83 (m, 2H); MS (electrospray$^-$) *m/e* 419 (M+H). Anal. Calcd for $C_{23}H_{20}N_2O_4S \cdot 0.75H_2O$: C, 63.85; H, 4.99; N, 6.45. Found: C, 63.57; H, 4.74; N, 6.74.

Example 6: Antibacterial Testing

The crude compounds were screened in a representative high throughput screening assay for antibacterial activity, and compounds **5-n-ii** and **5-n-vi** were found to have activities minimum inhibitory concentrations (MIC's) of 0.7-1.5 μM and 3-6 μM against *E. coli*, respectively. This activity was verified by manual solution synthesis of analytically pure material as described in Example 5 above, which had identical activity.

What is claimed is:

1. A method of preparing a library of compounds comprising:

selecting *in silico* a group of related fragments, each of said fragments constituting a part of said compounds, each of said related fragments having at least one attachment site;

selecting *in silico* at least one further fragment having at least one attachment site;

linking *in silico* said further fragment to said related fragments by connecting the attachment site of said further fragment to the attachment site of said related fragments to generate said virtual library of compounds;

generating synthesis instructions *in silico* for each of said members of said virtual library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

2. A method of preparing a library of compounds comprising:

selecting *in silico* a first fragment, said first fragment constituting a part of said compounds and having at least one attachment site;

selecting *in silico* a group of related fragments, each of said group of related fragments having at least one attachment site;

linking *in silico* each of said group of related fragments to said first fragment by connecting the attachment site of each of said group of related fragments to the attachment site of said first fragment to generate said virtual library of compounds;

generating synthesis instructions *in silico* for each of said members of said virtual library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

3. A method of preparing a library of compounds comprising:

selecting *in silico* a first group of related fragments, each of said first group of related fragments constituting a part of said compounds and having at least one attachment site;

selecting *in silico* a further group of fragments, each of said further group of fragments having at least one attachment site;

linking *in silico* each of said first group of related fragments to each of said further group of fragments by connecting the attachment site of each of said first group of related fragments to the attachment site of each of said further group of fragments to generate said virtual library of compounds;

generating synthesis instructions *in silico* for each of said members of said virtual library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

4. The method of claim 1 wherein each of said fragments is introduced *in silico* into said compounds by the use a corresponding reagent.

5. The method of claim 2 wherein each of said fragments is introduced *in silico* into said compounds by the use a corresponding reagent.

6. The method of claim 3 wherein each of said fragments is introduced *in silico* into said compounds by the use a corresponding reagent.

7. A method of preparing a library of compounds comprising:

dissecting said compounds into fragments *in silico*;

identifying each of said fragments in terms of a transformation wherein said transformation is a one to one link between the fragment and a reagent used to introduce said fragment into a compound;

using said transformations to generating synthesis instructions *in silico* for each of said members of said virtual library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

8. The method of claim 7 wherein said transformation is further associated with auxiliary reagents or reaction conditions.

9. A method of preparing a library of compounds comprising:

dissecting said compounds into fragments;

representing each of said fragments *in silico* as a transformation wherein each transformation is a one to one link between a fragment and a reagent used to introduce said fragment into one of said compounds;

selecting *in silico* a first group of said fragments, each of said first group of fragments constituting a part of said compounds, each of said first group fragments having at least one attachment site;

selecting *in silico* at least one further fragment having at least one attachment site;

linking *in silico* said further fragment to said first group of fragments by connecting the attachment site of said further fragment to the attachment site of said members of said first group of fragments to generate said virtual library of compounds;

generating synthesis instructions in silico for each of said members of said virtual library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

10. A method of preparing a library of compounds comprising:

dissecting said compounds into fragments;

representing each of said fragments *in silico* as a transformation wherein each transformation is a one to one link between a fragment and a reagent used to introduce said fragment into one of said compounds;

selecting *in silico* a fragment, said first fragment constituting a part of said compounds, said first fragment having at least one attachment site;

selecting *in silico* at group of further fragments each having at least one attachment site;

linking *in silico* said group of further fragments to said first fragment by connecting the attachment site of said group of further fragments to the attachment site of first fragment to generate said virtual library of compounds;

generating synthesis instructions *in silico* for each of said members of said virtual library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

11. A method of preparing a library of compounds comprising:

dissecting said compounds into fragments;

representing each of said fragments *in silico* as a transformation wherein each transformation is a one to one link between a fragment and a reagent used to introduce said fragment into one of said compounds;

selecting *in silico* a first group of said fragments, each of said first group of fragments constituting a part of said compounds, each of said first group fragments having at least one attachment site;

selecting *in silico* at group of further fragments each having at least one attachment site;

linking *in silico* at least some of the members of said group of further fragments to least some of members of said first group of fragments by connecting the attachment site of the members of said further fragments to the attachment site of said members of said first group of fragments to generate said virtual library of compounds;

generating synthesis instructions *in silico* for each of said members of said virtual library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

12. A method of preparing a library of compounds comprising:

dissecting said compounds into fragments;

adding said fragments together in sequential synthesis rounds;

tracking the addition of fragments of said compounds;

using the results of said tracking to generate synthesis instructions *in silico* for each of said members of said virtual library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

13. A method of preparing a library of compounds comprising:

dissecting said compounds into fragments;

representing each of said fragments *in silico* as a transformation wherein each transformation is a one to one link between a fragment and a reagent used to introduce said fragment into one of said compounds;

adding said transformations together in sequential synthesis rounds;

tracking said transformations *in silico* to generate synthesis instructions *in silico* for each of said members of said virtual library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

14. A method of preparing a library of compounds comprising:

dissecting each of said compounds into fragments;

linking together the fragments of each of the compounds;

tracking the sequence of linkage for each compound;

using the results of said tracking to generate synthesis instructions for each of said members of said library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

15. The method of claim 14 further including:

grouping two or more compounds of said library together to form a mixture; and

linking together the tracked information of each of the members of said mixture.

16. The method of claim 14 further including:

grouping two or more compounds of said library together to form a mixture;

grouping a further two or more compounds of said library together to form a further mixture;

linking together the tracked information of each of the members of said mixture; and

linking together the tracked information of each of the members of said further mixture.

17. A method of preparing a library of compounds comprising:

dissecting said compounds into fragments;

representing each of said fragments as a transformation wherein each transformation is a one to one link between a fragment and a reagent used to introduce said fragment into one of said compounds;

linking together the transformations of each of the compounds;

tracking the sequence of linkage for each compound;

using the results of said tracking to generate synthesis instructions for each of said members of said library; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

18. The method of claim 17 further including:

grouping two or more compounds of said library together to form a mixture; and

linking together the tracked information of each of the members of said mixture.

19. The method of claim 17 further including:

grouping two or more compounds of said library together to form a mixture;

grouping a further two or more compounds of said library together to form a further mixture;

linking together the tracked information of each of the members of said mixture;

linking together the tracked information of each of the members of said further mixture;

using the results of said tracking to generate synthesis instructions for each of said members of said mixture and said further mixture; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

20. The method of claim 19 wherein each of said members of said mixture are prepared together in one reaction vessel and each of said members of said further reaction mixture are prepared together in a further reaction vessel.

21. The method of claim 17 further including:

defining each said transformation to further include information related to the synthesis of its fragment from its reagent.

22. A method of preparing members of a library of compounds comprising:

grouping two or more compounds of said library together to form a mixture;

dissecting each of said compounds of said mixture into fragments;

linking together the fragments of each of the compounds of said mixture;

tracking the sequence of linkage of the members of said mixture;

using the results of said tracking to generate synthesis instructions for each of said members of said mixture; and

using said synthesis instructions to prepared said members of said mixture on an automated synthesizer.

23. A method of preparing a library of compounds comprising:

dissecting each of said compounds into fragments;

grouping said compounds of said library into mixtures where each mixture includes two or more member compounds of said library;

linking together the fragments of each of the compounds;

tracking the sequence of linkage of the members of each said mixture;

using the results of said tracking to generate synthesis instructions for each of said members of said mixtures; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

24. A method of preparing members of a library of compounds comprising:

grouping two or more compounds of said library together to form a mixture;

dissecting each of said compounds of said mixture into fragments;

representing each of said fragments as a transformation wherein each transformation is a one to one link between a fragment and a reagent used to introduce said fragment into one of said compounds;

linking together the transformations for each of the compounds of the mixture;

tracking the sequence of linkage of the members of said mixture; using the results of said tracking to generate synthesis instructions for each of said members of said mixture; and

using said synthesis instructions to prepared said mixture on an automated synthesizer.

25. The method of claim 24 further including:

defining each said transformation to further include information related to the synthesis of its fragment from its reagent.

26. A method of preparing a library of compounds comprising:

dissecting each of said compounds into fragments;

representing each of said fragments as a transformation wherein each transformation is a one to one link between a fragment and a reagent used to introduce said fragment into one of said compounds;

grouping said compounds of said library into mixtures where each mixture includes two or more member compounds of said library;

linking together the transformation for each of the compounds;

tracking the sequence of linkage of the members of each said mixture;

using the results of said tracking to generate synthesis instructions for each of said members of said mixture; and

using said synthesis instructions to prepared said compounds on an automated synthesizer.

27. The method of claim 25 further including:

defining each said transformation to further include information related to the synthesis of its fragment from its reagent.

28. A method of preparing a library of compounds comprising:

dissecting each of said compounds into fragments;

grouping two or more compounds of said library together to form a mixture;

linking together the fragments of each of the compounds;

tracking the sequence of linkage of the compounds;

using the results of said tracking to generate synthesis instructions for each of said compounds;

using said synthesis instructions to prepared said compounds on an automated synthesizer; and

preparing said members of said mixture in a common vessel.

29. A method of preparing a library of compounds comprising:

dissecting each of said compounds into fragments;

representing each of said fragments as a transformation wherein each transformation is a one to one link between a fragment and a reagent used to introduce said fragment into one of said compounds;

grouping two or more compounds of said library together to form a mixture;

linking together the transformations for each of the compounds;

tracking the sequence of linkage of the compounds;

using the results of said tracking to generate synthesis instructions for each of said compounds;

using said synthesis instructions to prepared said compounds on an automated synthesizer; and

preparing the members of said mixture in a common vessel.

30. A method of preparing a plurality of mixtures of compounds comprising:

selecting a plurality of compounds;

dissecting each of said compounds into fragments;

grouping said compounds into mixtures where each mixture includes two or more member compounds;

linking together the fragments of each of the compounds;

tracking the sequence of linkage of the members of each said mixture;

using the results of said tracking to generate synthesis instructions for each of said members of said mixtures;

using said synthesis instructions to prepared said mixtures on an automated synthesizer; and

preparing each mixture in its own reaction vessel.

31. A method of preparing a plurality of mixtures of compounds comprising:

selecting a plurality of compounds;

dissecting each of said compounds into fragments;

representing each of said fragments as a transformation wherein each transformation is a one to one link between a fragment and a reagent used to introduce said fragment into one of said compounds;

grouping said compounds into mixtures where each mixture includes two or more of said plurality of compounds;

linking together the transformation for each of the compounds;

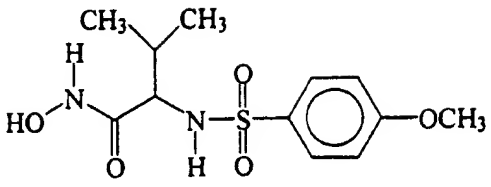
tracking the sequence of linkage of the members of each said mixture;

using the results of said tracking to generate synthesis instructions for each of said members of said mixture;

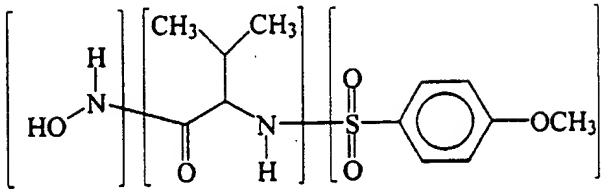
using said synthesis instructions to prepared said mixtures of compounds on an automated synthesizer; and

preparing each mixture in its own reaction vessel.

Compound CI



$C_{12}H_{18}N_2O_5S$



	F_i	F_{ii}	F_{iii}
Molecular formula	H_2NO	C_5H_9NO	$C_7H_7O_3S$

Figure 1

Addition of fragments to yield compounds

Table

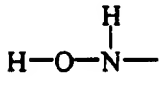
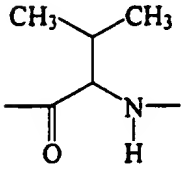
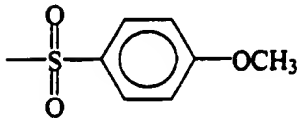
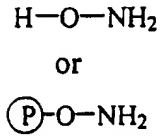
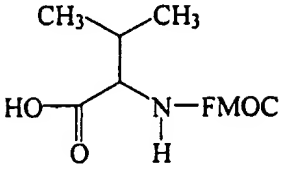
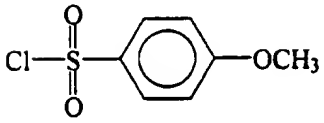
Fragment Identifier	Structure	Name	Molecular formula	Other
F_i		Hydroxylamine	H_2NO	...
F_{ii}		Amino acid	C_5H_9NO	...
F_{iii}		Sulfonyl	$C_7H_7O_3S$	...

Figure 2

Reagents	Identifier	Name	Properties
	R_i	Hydroxylamine	...
	R_{ii}	FMOC blocked amino acid	...
	R_{iii}	Sulfonylchloride	...

Ⓟ = Solid support

Figure 3

Transformation

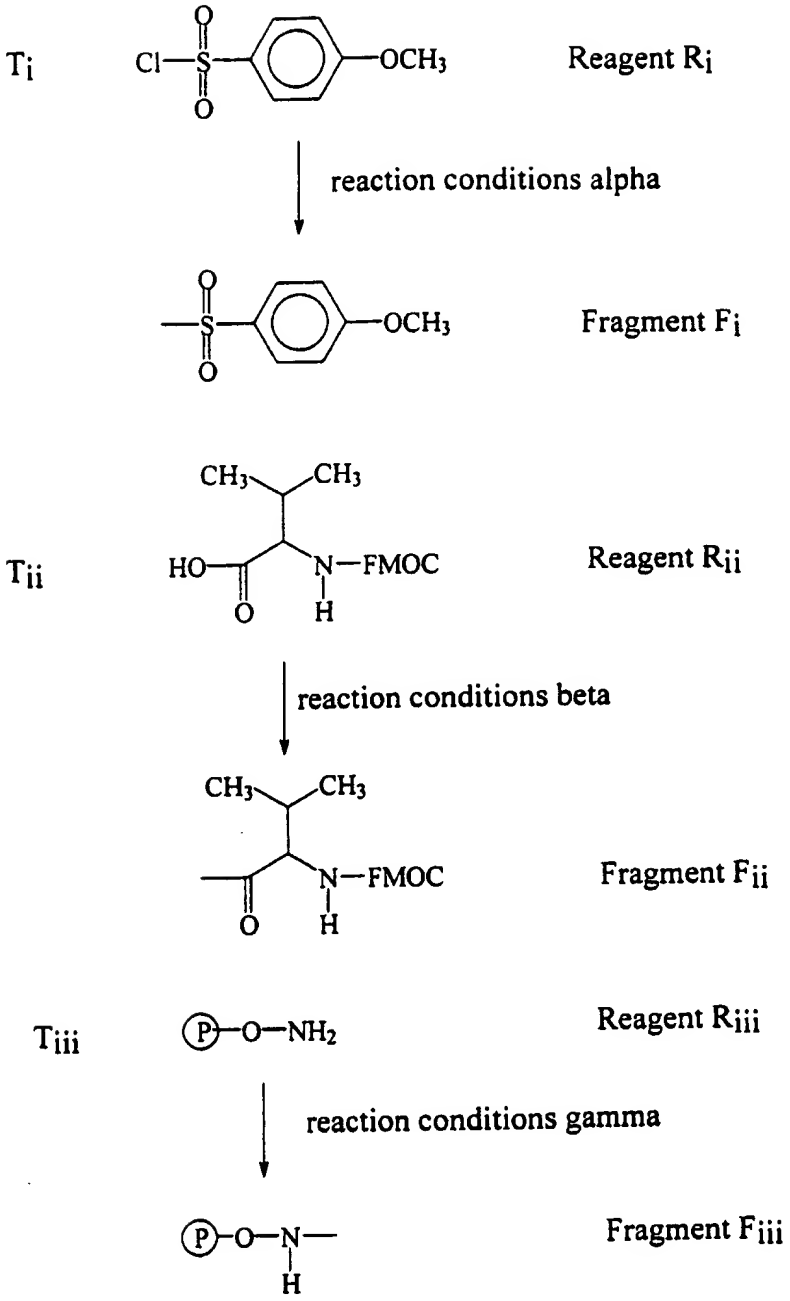


Figure 4

Common Fragment / Different Reagents and Transformations

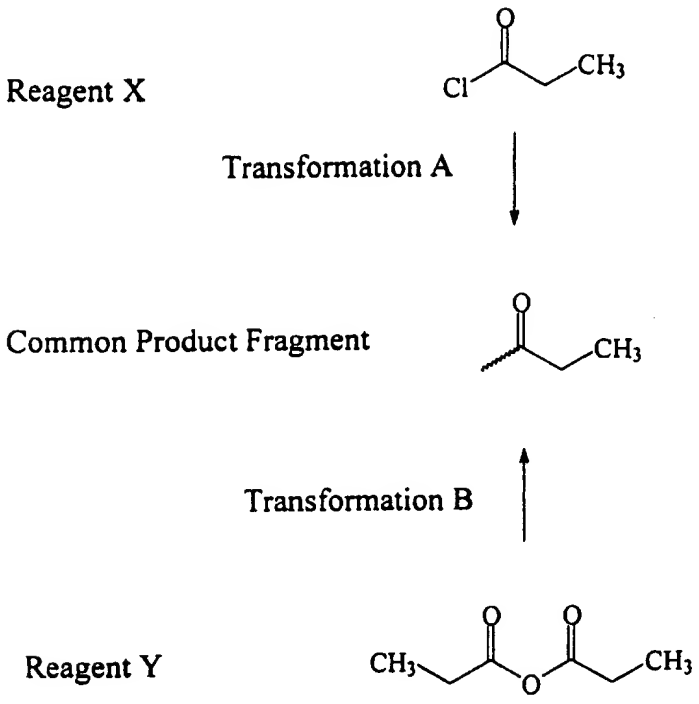


Figure 5

Common Fragment / Different Reagents and Transformations

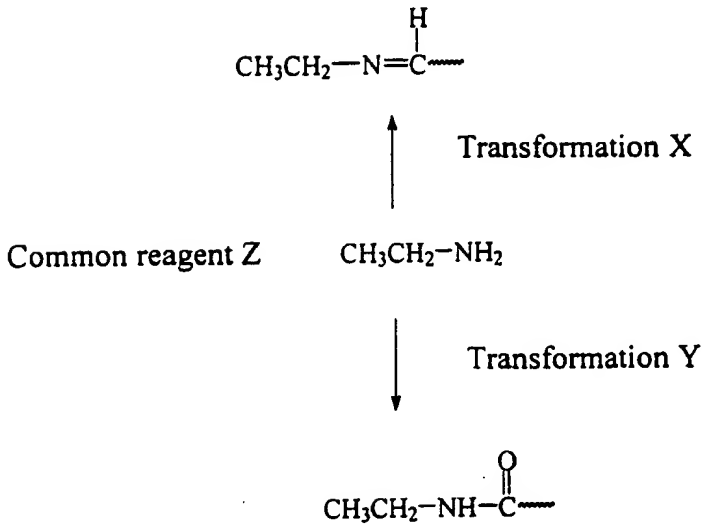


Figure 6a

Common Reagent

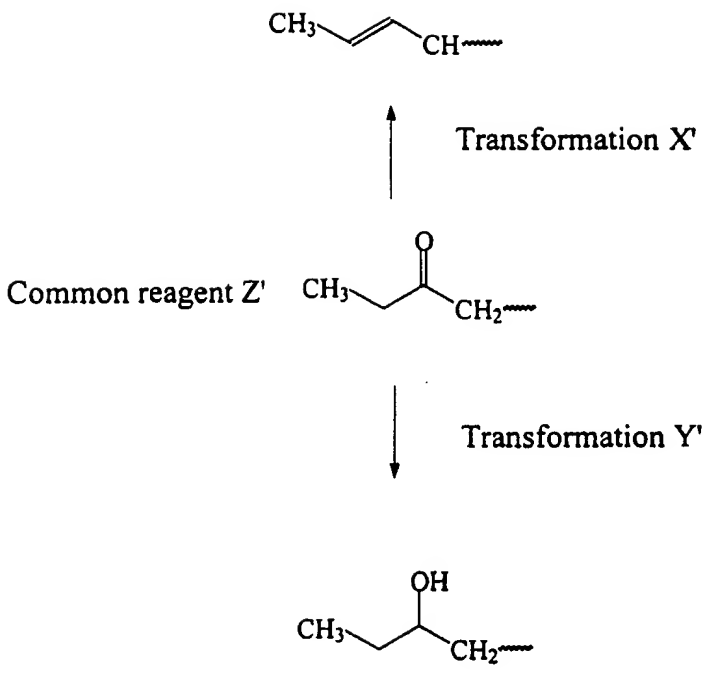
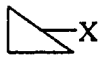
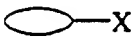
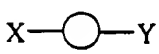
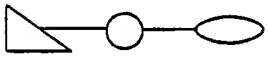


Figure 6b

Symbolic addition of fragments to yield compound

Symbolic Structure	Symbolic Identifier	Molecular formula
Fragment		
	$F_{i'}$	$C_uH_vN_w$ ....
	$F_{ii'}$	$C_uH_vN_w$ ...
	$F_{iii'}$	$C_uH_vN_w$ ...
Compound		
	CI'	$C_uH_vN_w$ ...

$$\text{Molecular formula } F_{i'} + \text{Molecular formula } F_{ii'} + \text{Molecular formula } F_{iii'} = \text{Molecular formula CI'}$$

Figure 7

Symbolic Reagent Table

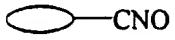
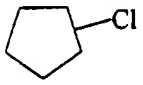
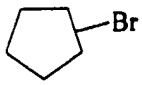
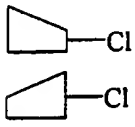
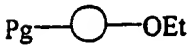
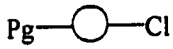
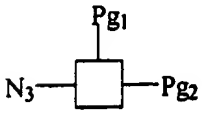
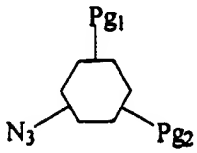
Identifier	Name	Structure	Molecular formula
R1	xxx		xxx
R2	...		...
R3	...		...
R4	...		...
R5	...		...
R6	...		...
R7	...		...
R8	...		...
R9	...		...
R10	...		...

Figure 8

Symbolic Fragment Table

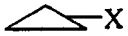
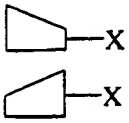
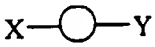
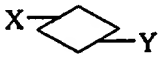
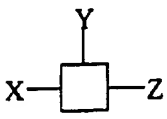
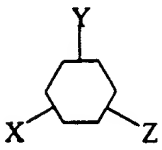
Identifier	Symbolic Structure	Molecular formula	Molecular Weight
F1		xxx	xxx
F2		...	...
F3		...	...
F4		...	...
F5		...	...
F6		...	...
F7		...	...
F8		...	...

Figure 9

Symbolic Transformation Table

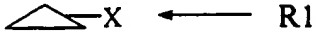
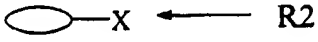
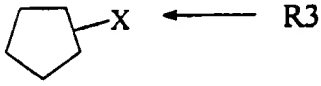
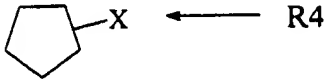
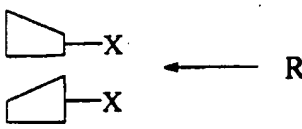
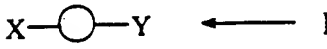
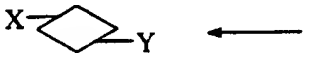
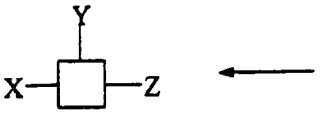
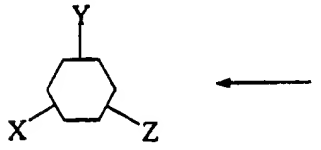
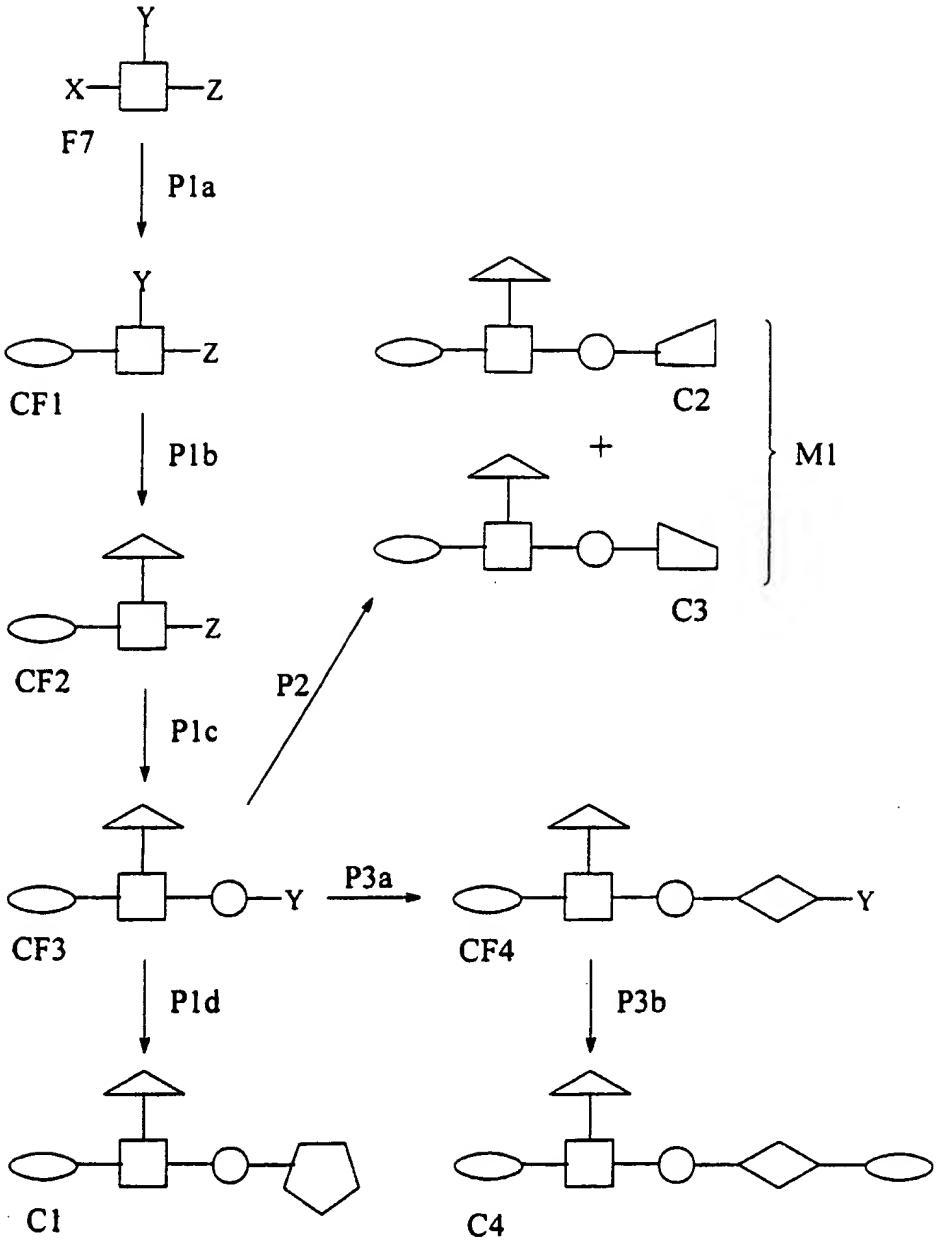
Identifier		Symbolic Reactions	Reagent
T1	F1		conditions α
T2	F2		conditions β
T3	F3		conditions α
T4	F3		conditions α
T5	F4		conditions α
T6	F5		conditions ε
T7	F5		conditions α
T8	F6		conditions α
T9	F7		conditions γ
T10	F8		conditions γ

Figure 10

Single Compounds and Mixtures



P = synthetic path CF = complex fragment
F = fragment M = mixture
C = compound

Figure 11

Mixture 2

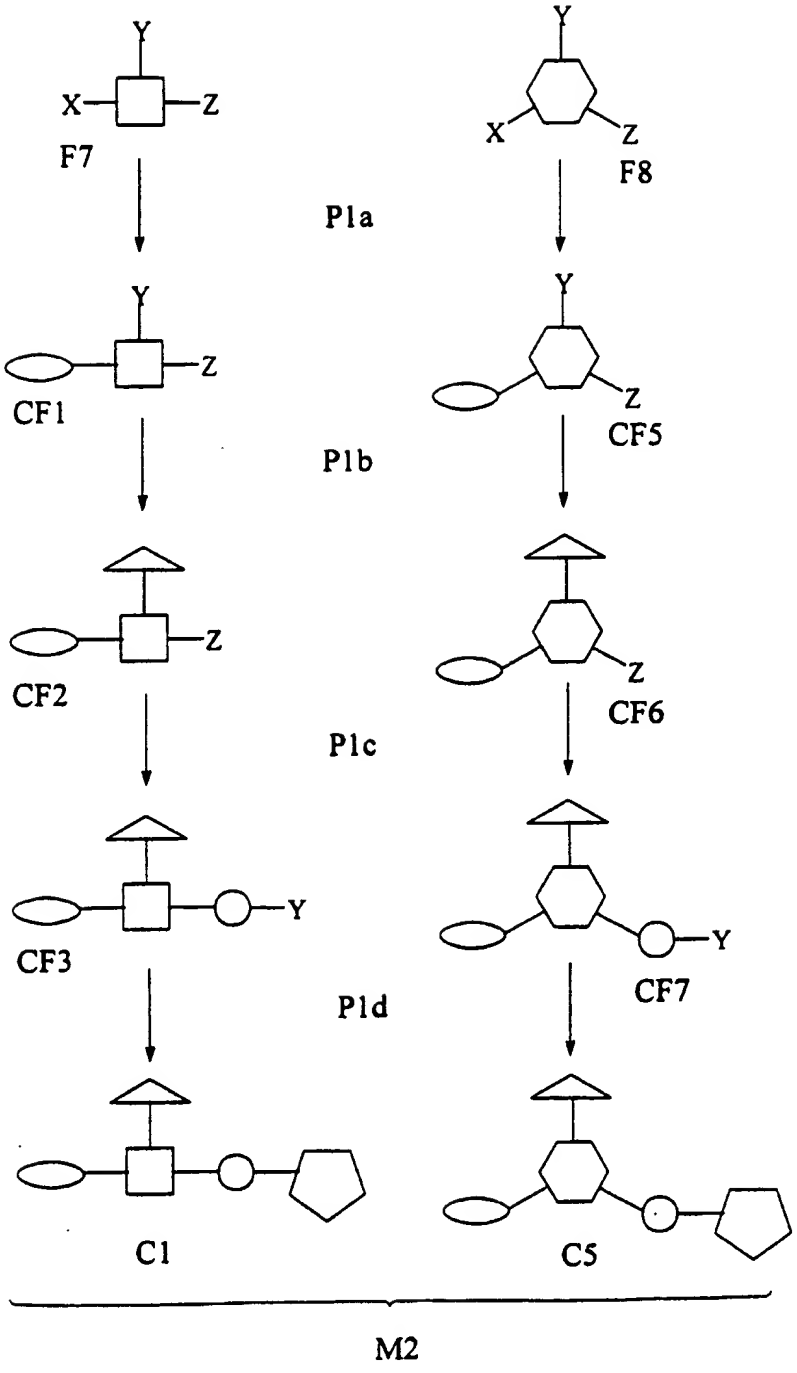


Figure 12

Mixture 3

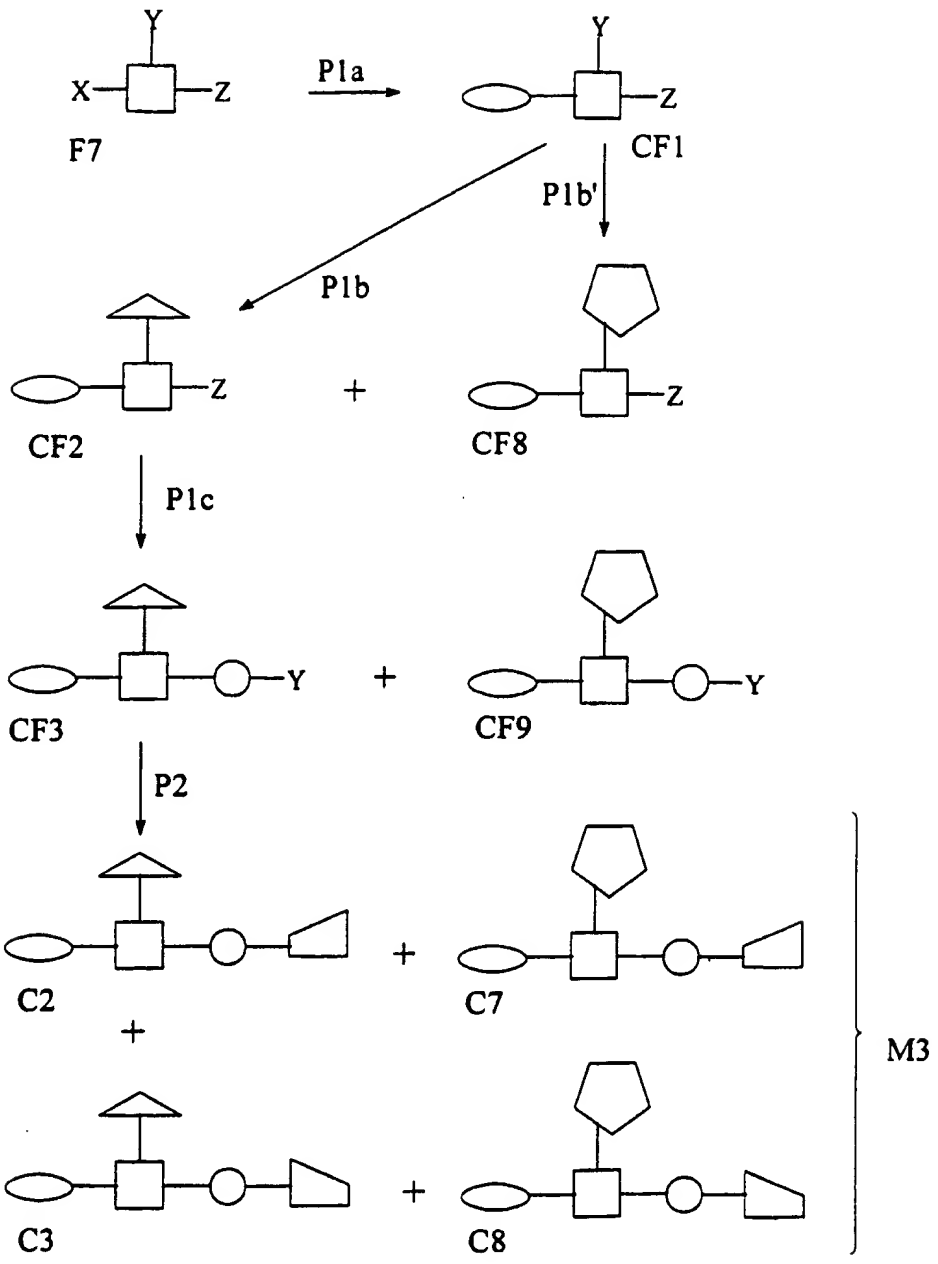
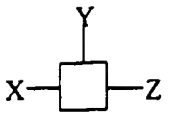


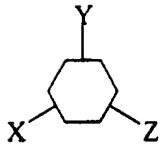
Figure 13

Mixture 4

2 Starting Fragments

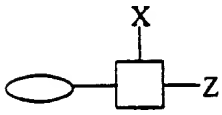


F7

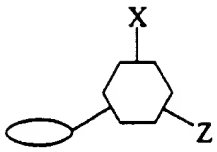


F8

2 Complex Fragments

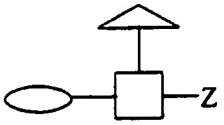


CF1

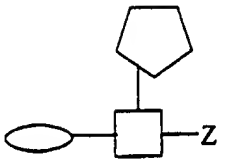


CF5

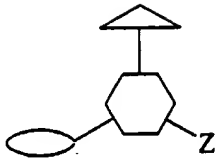
4 Complex Fragments



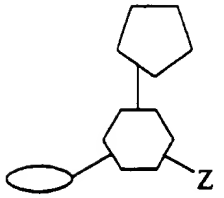
CF2



CF8

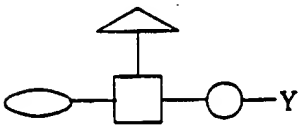


CF 6

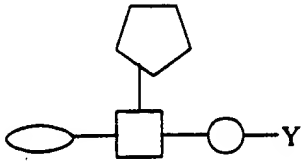


CF11

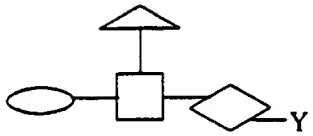
8 Complex Fragments



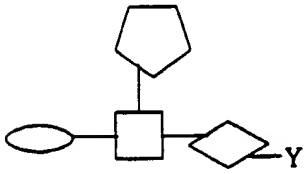
CF3



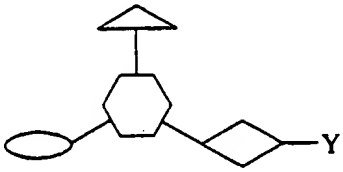
CF9



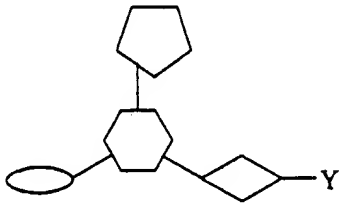
CF12



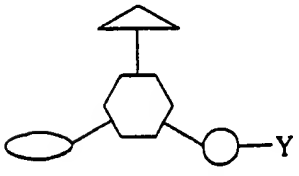
CF13



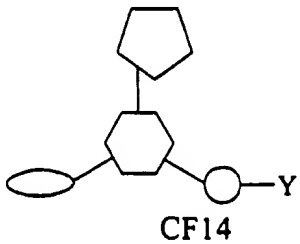
CF15



CF16



CF 7



CF14

Figure 14a

Mixture 4 (continued)

16 compounds

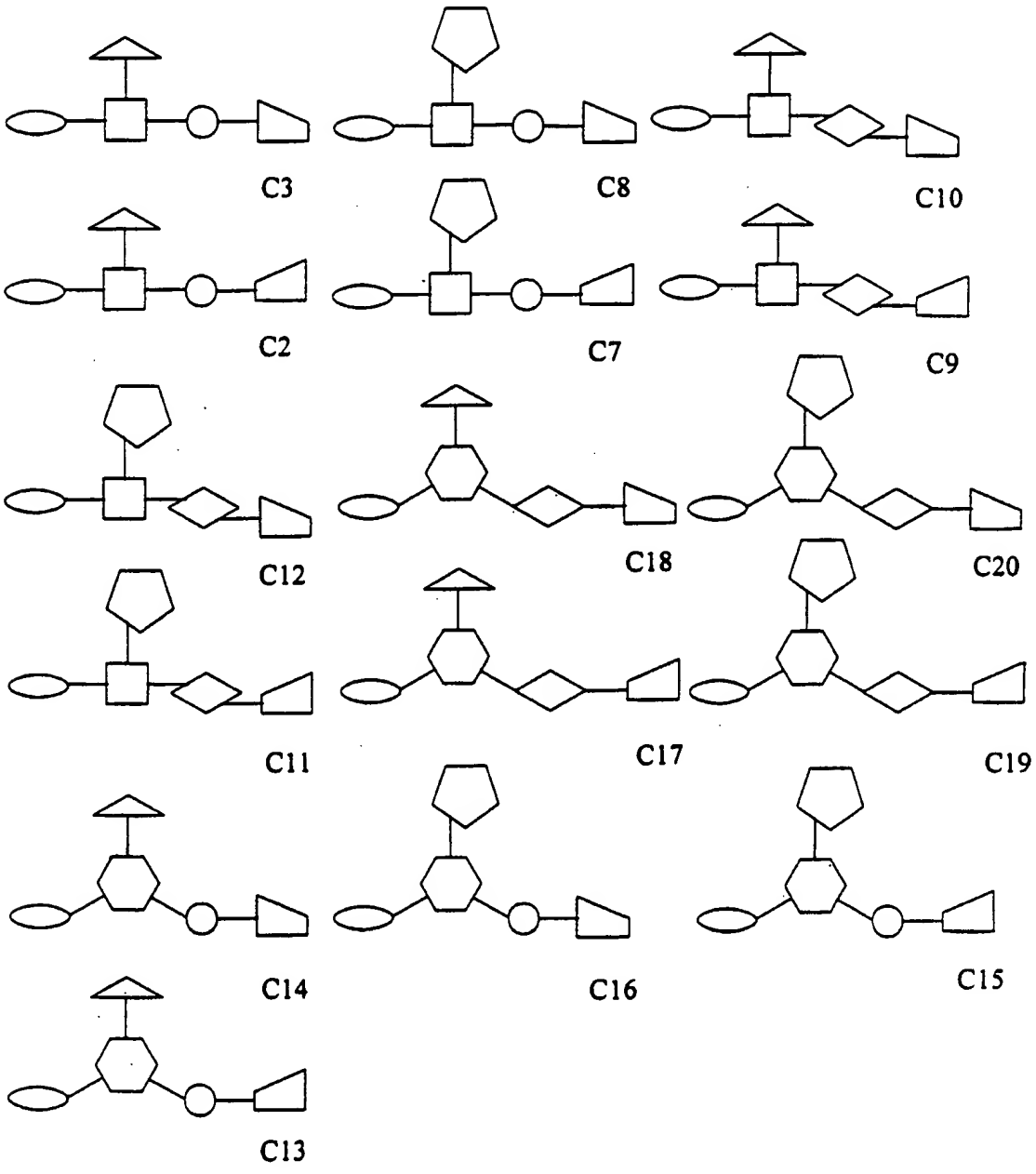


Figure 14b

Tracking Table for Compound C1

(a) By Fragments:

n	n+1	n+2
F7	F2	
	F1	
	F5	
		F3

(b) By Transformations:

Synthesis Path 1

n	n+1	n+2
T9	T2	
	T1	
	T6	
		T3

Synthesis Path 2

n	n+1	n+2
T9	T2	
	T1	
	T7	
		T3

Synthesis Path 3

n	n+1	n+2
T9	T2	
	T1	
	T6	
		T4

Synthesis Path 4

n	n+1	n+2
T9	T2	
	T1	
	T7	
		T4

Figure 15

Tracking Table

Tracking M1

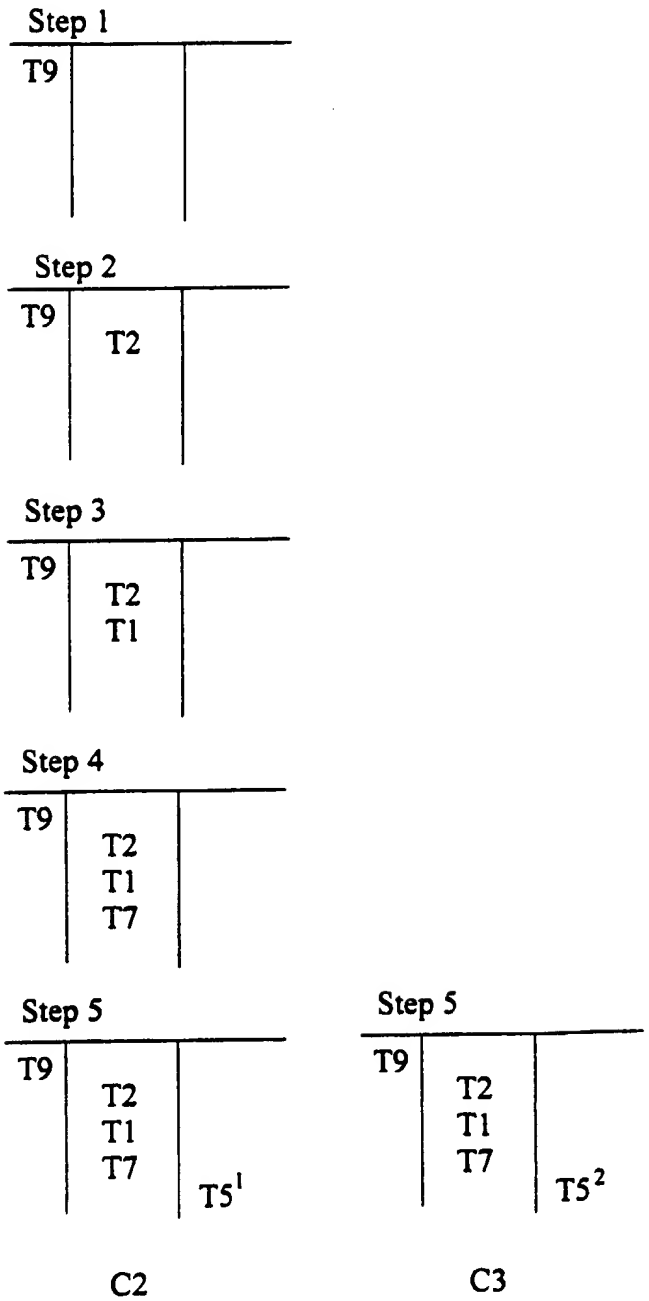


Figure 16

Tracking Table

Tracking M2

Step 1		
n	n+1	n+2
T9		

Step 1		
n	n+1	n+2
T10		

Step 2		
n	n+1	n+2
T9	T2	

Step 2		
n	n+1	n+2
T10	T2	

Step 3		
n	n+1	n+2
T9	T2 T1	

Step 3		
n	n+1	n+2
T10	T2 T1	

Step 4		
n	n+1	n+2
T9	T2 T1 T7	

Step 4		
n	n+1	n+2
T10	T2 T1 T7	

Step 5		
n	n+1	n+2
T9	T2 T1 T7	T4

Step 5		
n	n+1	n+2
T10	T2 T1 T7	T4

C1

C5

Figure 17

Tracking Table

Tracking M3

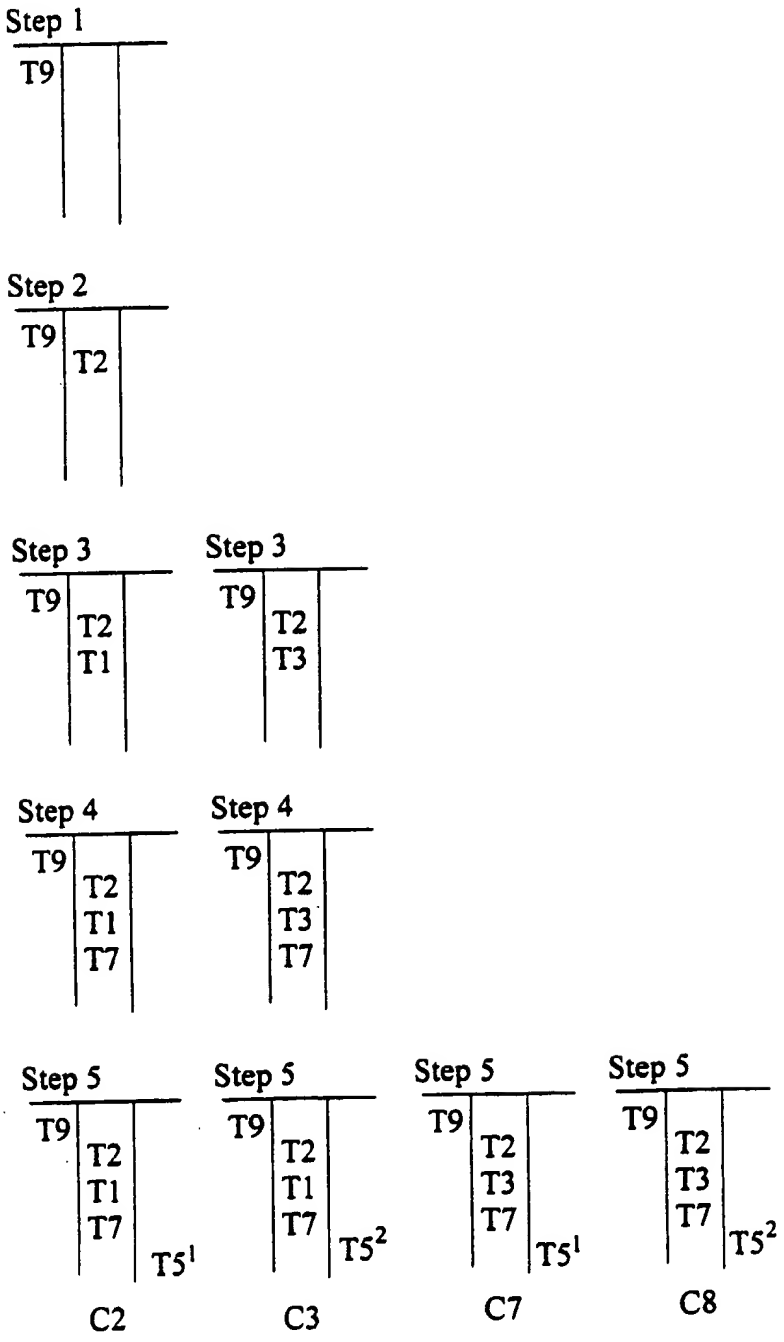


Figure 18

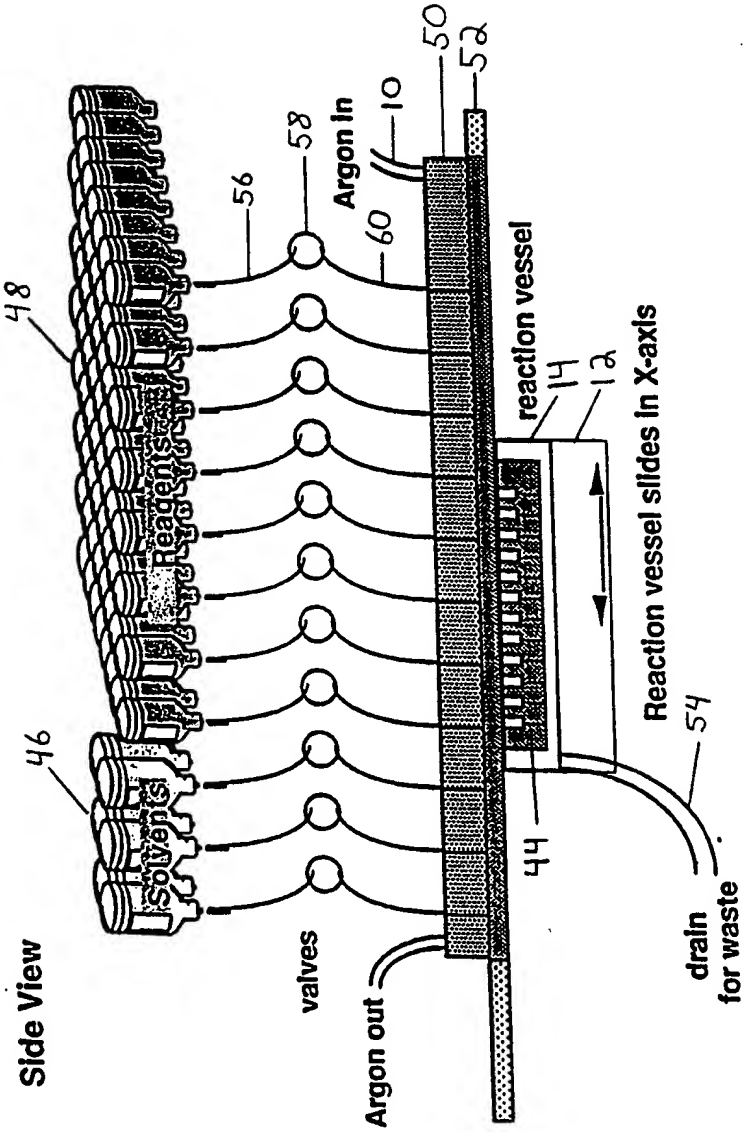

Side View
Solvents
Reagents
46
48
56
58
Argon in
60
10
50
52
reaction vessel
14
12
Reaction vessel slides in X-axis
54
44
valves
Argon out
drain
for waste

Figure 19

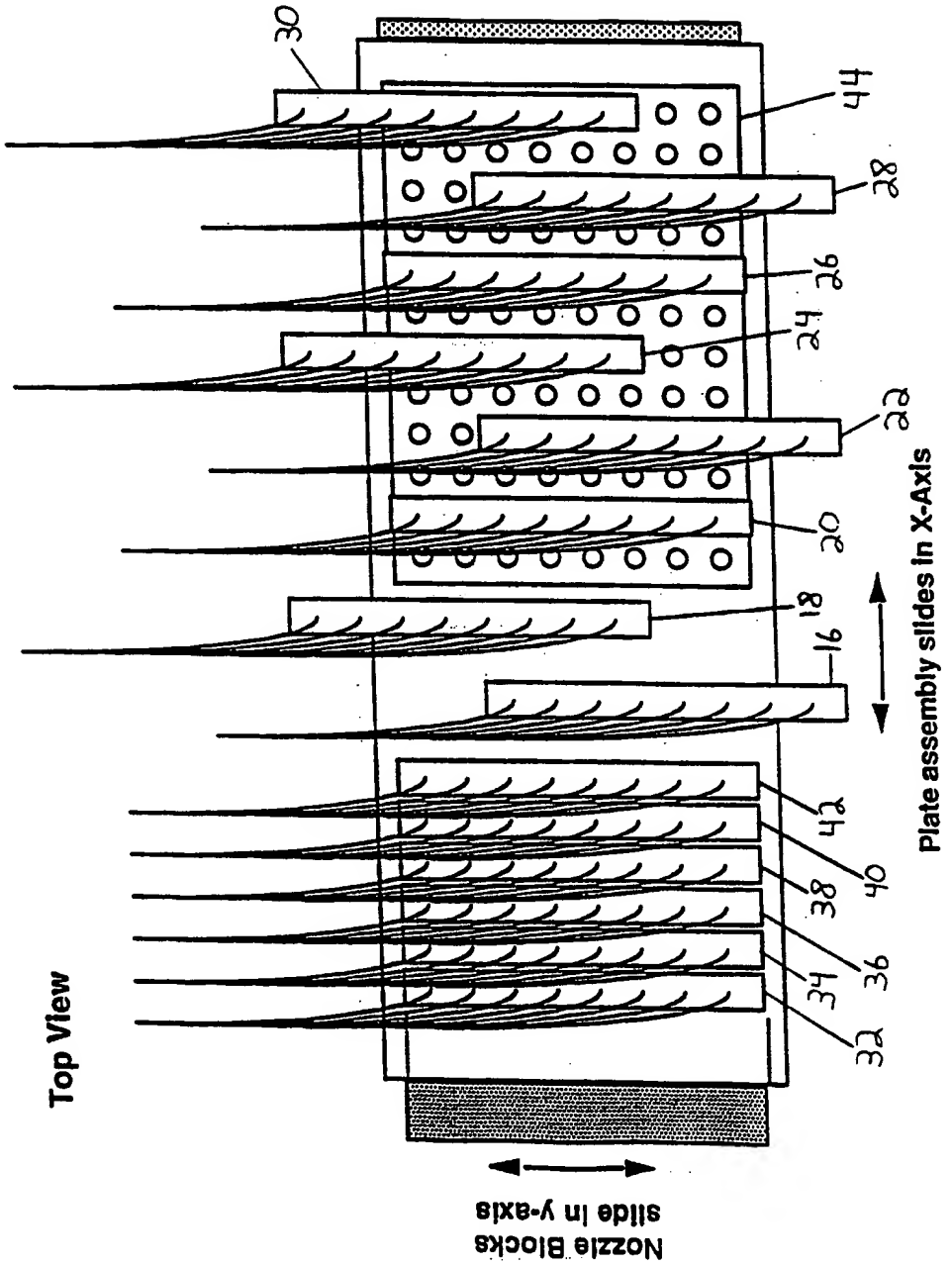

30
44
28
26
24
22
20
18
16
42
40
38
36
34
32
Top View
Plate assembly slides in X-Axis
Nozzle Blocks
slide in y-axis

Figure 20

Synthesis of hydroxamic acids from alcohol resin

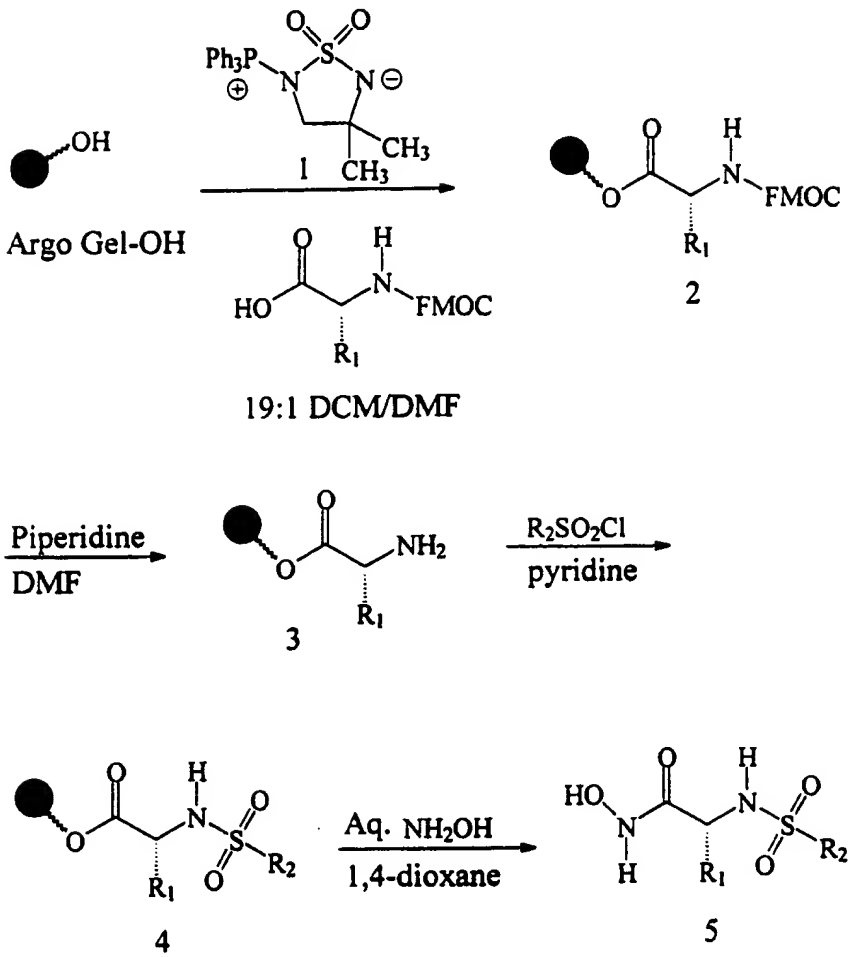


Figure 21

Synthesis of hydroxamic acids from hydroxylamine resin

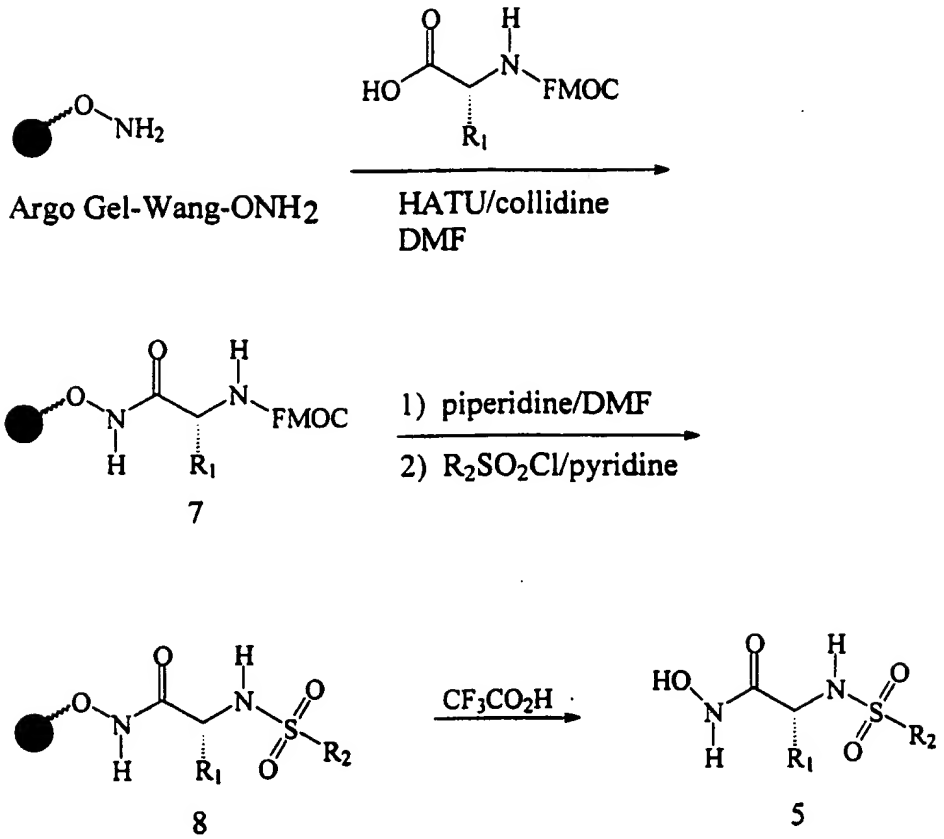


Figure 22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10383

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C06B 1/OO ; CO7K 1/04 ; C12Q 1/00; G01N 33/566

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4; 436/501

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DERWENT, DIALOG
search terms computer, program, software, predict synthesis, retrosynthetic, antithetic, fragments,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,703,792 A (CHAPMAN) 30 December 1997, see entire document.	1-31
Y	US 5,574,656 A (AGRAFIOTIS et al) 12 November 1996, see entire document.	1-31
Y	US 5,434,796 A (WEININGER) 18 July 1995, see entire documnet.	1-31
Y	COREY et al., Computer-Assisted Analysis in Organic Synthesis. Science. 26 April 1985, Vol. 228, No. 4698, pages 408-418, see entire document, note the references to retrosynthetic or antithetic synthesis.	1-31

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
04 AUGUST 1999

Date of mailing of the international search report
10 SEP 1999

Name and mailing address of the ISA/US
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Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10383

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROTHSTEIN et al. GroupBuild: A fragment-based method for de novo drug design. J. Med. Chem. 1993, Vol. 36, No. 12, pages 1700-1710.	1-31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10383

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/4; 436/501